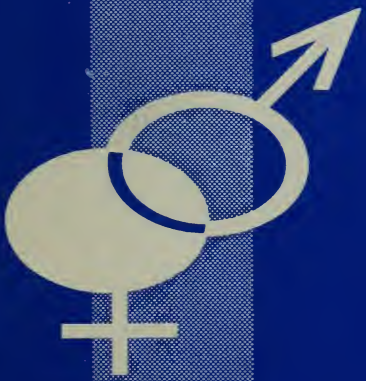
PROMISE OF THE
Life Sciences



U. S. DEPARTMENT OF AGRICULTURE-GRADUATE SCHOOL

PROMISE OF THE
Life Sciences

Edited by
Marguerite Gilstrap

U. S. DEPARTMENT OF AGRICULTURE·GRADUATE SCHOOL

UNITED STATES DEPARTMENT OF AGRICULTURE
GRADUATE SCHOOL

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PREFACE

This book, "Promise of the Life Sciences," brings together lectures given by five scientists in a series in Jefferson auditorium of the U. S. Department of Agriculture in the autumn of 1960.

The series was arranged by the USDA Graduate School, a self-supporting institution that since 1921 has carried on an educational program for Federal employees.

Along with free public lectures the School offers each year more than 300 evening courses, a small correspondence program, and special institutes and seminars to supplement in-service training. Most of our teachers hold full-time government jobs as do the members of the committees that plan our curriculum and other activities.

Plans for the lecture series, "Promise of the Life Sciences," were drawn up by a committee of scientists under the chairmanship of Dr. Byron T. Shaw, Administrator of the Agricultural Research Service. Assisting him were: Dr. Theodore C. Byerly, Dr. Sterling B. Hendricks, and Dr. Erwin L. LeClerg of the Agricultural Research Service, Dr. Graham P. DuShane, editor of SCIENCE, Dr. Sidney R. Galler, Office of Naval Research, and Dr. Robert Kelman, Executive Office of the President. I served *ex officio.*

A grant from the National Science Foundation enabled us to record the lectures on videotape and 16-mm film. The master videotapes have been placed in the Library of the National Educational Television and Radio Center for distribution to educational television stations. Copies of the films have been distributed to selected film libraries and the regional libraries of the U. S. Department of Agriculture motion picture service. The Graduate School has also retained copies of the films for rent and for sale.

We are indebted to many people who helped us present the lecture series. Among those who should be mentioned particularly are Thomas F. McGinty, coordinator of the lectures and his associates in the information division of the Agricultural Research Service, and Dr. Paul Shaefer and his colleagues in the television division of the Walter Reed Army Medical Center.

Marguerite Gilstrap, the editor of this book, has been assisted by Alice Lane, Helen McConkey, Maxine Gardner, and F. A. Schaeffer of the information division of the Agricultural Research Service. Miss Lane designed the cover. Miss McConkey and Mrs. Gardner transcribed the lectures and typed the manuscript, and Mr. Schaeffer drew up specifications for the printer.

JOHN B. HOLDEN
Director of the
USDA Graduate School

INTRODUCTION

Recent events suggest that great discoveries in biology are imminent.

We cannot say precisely when the discoveries will be made. But we can be sure they will have profound effects upon applied biology—upon medicine, natural biology and agriculture. These basic discoveries will enable man to produce and enjoy agricultural products in increasing amounts and variety.

We asked the distinguished scholars in this lecture series to tell us about advances at the frontiers of the research in which each of them specializes. Taken together, the lectures cover spectacular gains across the broad range of the life sciences.

In agricultural research we are drawing heavily on this accumulating store of knowledge. And we are contributing to it. This mutually helpful exchange is not new. It has been going on for nearly 75 years.

One of the first important contributions of agricultural research to the life sciences was made about 1890. It showed the role of insects in the transmission of disease. It was immediately put to use to eradicate tick fever in cattle. The discovery helped to pave the way for modern man to conquer terrible human ills—malaria, yellow fever, typhus, and bubonic plague.

Today, agricultural scientists—along with biological research workers around the world—are doing research at the molecular level. They are seeking to understand the facts of nature and formulate great natural laws that will have broad and continuing application.

Each scientist who hears or reads these lectures will relate the information to his own specialty. I will point, briefly, to some of the implications for agricultural research.

First, *the biological transformation of energy:* Life depends on the storage of solar energy by green plants and on the transformation of energy by microbes and animals, including man, into living tissues and other products. More efficient use of energy by crops and livestock is one of the goals of agricultural research. Plants vary widely in efficient storage of energy. We have highly efficient dwarf wheats under test that yield five times as much as the wheats ordinarily grown. Space studies have identified algae that store energy far in excess of other species. The findings suggest that yields of stored energy far above those now realized may some day be commonplace.

In animals, losses from the gross energy in plant materials fed may reach 65 percent in deficient diets. Nearly a third of the energy is used just to keep the animal warm. Basic biological research may show us ways to use more of the stored plant energy in animal feeds. We were able, through research, to reduce by half the nutrients required to produce a pound of live broiler. Perhaps we can do the same for cattle and other livestock.

Second, *genetics:* Genetics cuts across all of the biological sciences. Among the most impressive areas of advance in recent biological research is that of the nature of the gene.

Genetic research within the past decade, has identified DNA as the essential carrier of life, the enzyme proteins as the cell's working tools, and RNA as the channel of communication between them. The synthesis of DNA with specific genetic properties appears to be on the horizon. More knowledge of the precise and intricate interrelations between DNA, RNA, and the enzyme proteins will have a bearing on many lines of agricultural research. It will facilitate progress in studies of the transfer of resistance from one species of crop plant to another, of gene-to-gene relationships between a host and its parasite, and many other questions.

Third, *nutrition—the chemistry of life:* Today, we have only a

partial understanding of the metabolic pathways, the enzyme systems, and the metabolites in animal energy. Several aspects of nutrition bear on the partition of energy for building meat, milk, and eggs, for storing fat, and for work. These include the calorie-protein ratio in the diet, the total energy uptake, and the vitamins and other helpful nutrients.

Do variations in quantity and quality of these sources of energy affect health and productivity of livestock? Of man? Can man avoid the effects of old age by limiting his intake of cholesterol? Opinions vary. The research on which they are based is limited. Our wide experience with high energy diets for livestock shows no adverse effects on animal health. The Department's nutrition research indicates that the intake and source of energy foods can be evaluated only in relation to other dietary factors.

Fourth, *fine structure and pattern of living things:* Research in electron microscopy, biochemistry, and microbial genetics is giving us a much more detailed and precise map of the make-up of living matter. Cytologists are going beyond the structure of killed cells to an understanding of the functions and areas of activity within and among cells. Molecular biology may reveal how normal function is established and maintained in the organism at all stages of growth. This is an important step toward the understanding of normal development, wound repair, regeneration, and malignancy.

Fifth, *the organism and the evironment:* Perhaps the most difficult, most imperfectly understood, and most promising area of biology is that of the relationship of the organism to its environment. New leads have come from recent studies of biological orientation, biological clocks, imprinting of new born animals, territorialism in birds and plants, the effects of microclimate, and ways of adapting the macroclimate to the needs of biological species. Each of these leads promises greater discoveries to come. Studies of the relationships between host and parasite have already

been highly productive—in basic knowledge of immunogenetics, antibiotics, biological control of insects and other pests. There is more to come, much more.

The high promise of the life sciences is that the biological nature of life itself may be discovered and defined. Man must learn to use biological knowledge to plan his own destiny. His future depends on it.

Byron T. Shaw
Administrator
Agricultural Research Service

BIOLOGICAL TRANSFORMATION OF ENERGY

By Albert Szent-Györgyi

One of the most exciting chapters of modern biological research has to do with the problem of energy and how it drives life and builds up its substance.

Our bodies are built of substances consisting chiefly of carbon, hydrogen, nitrogen and oxygen, C, H, N and O, to use the chemical shorthand. All these elements are present in water and air. To build them into living machinery we need a mechanism which puts them together in the right order. Energy is needed to put them together in this new way, and drive the machinery thus constructed.

The ultimate source of all the energy building and driving life is reaching our globe in the form of the radiation of the sun. It sends to us its light rays, tiny packages of energy called "photons" which have the mysterious quality of behaving, at the same time, as little bullets and waves. If such a photon hits a molecule on our globe, and interacts with it, it raises, "excites," one of the electrons of this molecule to a higher energy level.

Molecules are structures built of atoms. Atoms are built of a nucleus and a surrounding cloud of electrons, tiny electric particles. These particles are arranged within the atom in pairs on different energy levels which we usually symbolize by horizontal lines. An electron, excited by the photon to a higher energy level, falls back

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Albert Szent-Györgyi carries on his notable studies of the physiology and biochemistry in muscle action at the Institute for Muscle Research, Woods Hole, Mass. He came to the United States in 1947 after a distinguished career in teaching and research at the universities of Szeged and Budapest, Hungary. He received the Nobel prize in medicine for 1937.

to its original level as a rule, within a very short period of about one hundred millionth of a second.

Life has learned to catch the electron in its high-energy-state and let it drop back to its original level through its own machinery, utilizing the energy thus released for its own purposes.

The question of how life utilizes the electronic energy to build up itself, and to have itself driven by it, is one of the most fundamental and exciting problems of modern biology.

Science has studied for a great many years what happens in the green leaves of the plants which catch the radiation of the sun with their green dye, chlorophyll. This research has led to many interesting and important discoveries but has left us without a really deep insight. We could fully understand the underlying reactions if we could go back right to the origin of life and see how the living world developed, gradually, from inanimate matter to its present high degree of development. Unfortunately, we cannot do this. What we can do is to go as far back towards the origin of life as possible by using the simplest living creatures for our study. The leaves of the green plant, already, represent a very late and high degree of development.

A very important step has been made in this direction, during the last decade, by shifting from the study of green leaves to the study of photosynthetic bacteria, which are much closer, possibly millions of years closer, to the origin of life. These bacteria contain tiny granules, chromatophores, containing the same substance, chlorophyll, which gives the green color to the leaves. Chlorophyll has a complicated chemical structure. It is built so that electrons can move freely through its molecule.

This substance is, in a way, the foundation on which life is built. We have no chlorophyll, ourselves, in our bodies, but we know that the energy transformations in our bodies are linked to substances which are the closest relatives of chlorophyll. You have to change the structure of the chlorophyll molecule only slightly to obtain the red hemoglobin that carries the oxygen in

our blood. Another small change will produce "cytochromes." These are closely related to hemoglobin and chlorophyll. They play a most important part in transporting electrons in our own cells and utilizing their energy.

We owe not only the energy, but also most of the beauty of our lives to these substances. The green of the forest and the rose color of the girls' cheeks and lips are all due to these closely related substances.

Now let me come back to our electrons and our photons and ask: what happens in these photosynthetic bacteria when an electron of this chlorophyll is raised to a higher energy level by a photon? Many laboratories are working on this problem.

Exciting new data come from the laboratory of Daniel Arnon in Berkeley. He has recently shown that these electrons, with their high energy, can produce hydrogen from water (probably its protons). Hydrogen is the main fuel, and also one of the main building stones of our bodies.

Another discovery has shown that the energy of these excited electrons can also be used directly to bind the nitrogen gas of the atmosphere. As you all know, nitrogen is one of the most important constituents of protein. So, the energy of the electron of chlorophyll, trapped in its excited state, can provide two of the main building blocks of the living machinery, H and N. The other two, C and O, are present, ready-made, in the air in the form of carbonic acid, CO_2, which can easily be bound by various chemical reactions.

Energy is needed, not only to provide these building blocks but also to hitch them together to bigger molecules, and, eventually, to maintain and drive the machinery thus constructed.

The trouble with sunshine is that it is periodic. The sun shines only in daytime, while life is continuous and needs energy all of the time. Excitation energies are very shortlived. They cannot be stored as such for long periods. For storage they have to

be converted into some more stable form, into chemical energy, that is, the energy of some chemical compound.

Nature must have discovered at an early date that this is possible by linking two phosphate molecules together. About 10 calories worth of energy is needed to link two phosphate molecules together. The energy can be liberated later by taking the two phosphates apart. Such a P–O–P link is thus essentially a little package of energy the chemist calls the "high energy phosphate bond," and symbolizes with a wriggle, "~".

When the excited electron drops back to its original "ground level" in the chromatophore of these photosynthetic bacteria, its energy is used to produce two such "high energy phosphate bonds" on a complex molecule, adenosine triphosphate (ATP). As the electron drops it passes through two substances, a "quinone" and a "cytochrome."

We can sum up the story, this far, by saying that while the electron goes around in its little circle, nothing is used but a photon and nothing is left but high energy phosphate links on ATP. This is what Arnon calls a "closed cycle."

At the side of this quinone-cytochrome cycle, another similar "closed cycle" has been discovered in which the electron may go around, leaving behind "~"-s. The substances figuring in this cycle are different. They are what we briefly call TPN, FMN (the vitamin B_2) and cytochromes. The "~"-s, that is, the ATP-s built in these cycles then could drive life with their energy also at night, when no radiation was available.

Nobody can tell us, today, what torturous paths Nature had to pass to develop these simple systems, nor can anybody tell us how many millions of years it took to develop them. Looking back from today's elevation, this seems an early and primitive development but these systems belong to the foundation on which our own lives are built.

When Nature perfects itself and develops new systems, it doesn't throw the old ones away but just improves them, or adds

to them. So, the quinone, the riboflavin, the cytochromes and the TPN are still the basic parts of which our own energy-producing machinery is composed. Some of you may remember I showed, many years ago, that ATP drives our muscles. As it moves us, it reflects developments that took place millions, or hundreds of millions of years ago.

The two cycles I have described could not support, in themselves, higher, more complex forms of life that are not only more complicated, but also more bulky. For many reasons, none of the substances I have mentioned—ATP, TPN, riboflavin, the quinones, cytochromes—can be stored in big quantities. Larger energy stores were needed to develop higher forms of life. External substances, present in great quantity, had to be used to establish these stores.

So the next dramatic chapter of the development of life relates to the use of two external substances present in Nature, in abundance: the one is water, the other is carbon dioxide. In this new step of development Nature has learned how to use the energy of sunshine, that is, the excitation energy of electrons, to decompose water, H_2O, into its elements, hydrogen and oxygen. This process takes place now in the green leaves of the plants. The oxygen thus formed is sent back into the atmosphere. We owe it to this reaction that our air contains oxygen. The hydrogen is linked to TPN. From the TPN this hydrogen is transferred stepwise onto CO_2. Once the cell has TPN–H and ATP, it can transfer the hydrogen from TPN–H onto the CO_2 and make out of it, carbohydrate.

$$n(4H) + n(CO_2) = nH_2O + nCnH_2nO_n$$

$C_nH_{2n}O_n$ is the general symbol of carbohydrate.

Without these reactions our atmosphere could contain no oxygen and higher life, as we know it, would be impossible. The carbohydrate is one of our main foodstuffs. It is also the material, which, in the form of cellulose, gives the solid structure to plants.

By adding more hydrogen to carbohydrate and taking off oxygens, the carbohydrate can be transformed into fat. Fat and carbohydrate, the main food stuffs, can be stored in practically unlimited quantity. What the plant does when it wants to utilize the energy of its fat or carbohydrate is to perform these reactions in the opposite direction; that is, take off the hydrogen, which it has put, earlier, onto the molecules, and transfer them back onto TPN. The TPN–H can easily exchange its H-s for electrons. What happens next is that these electrons are sent down from TPNH over FMN to the cytochromes.

The electrons pass, thus, the same cycle which I described before, producing, on their way, ~-s. The only difference is that eventually the electron does not go back from cytochrome to chlorophyll. It goes to the oxygen which the plant can pick out of the atmosphere. The oxygen then exchanges its electrons for H-s and forms H_2O, water.

This photosynthetic apparatus is a very bulky one and works very slowly, accumulating fats and carbohydrates. No very high and mobile forms of life could be developed by the methods described. The bulkiness of plants makes them immovable. But these reactions opened the way to the development of a new parasitic form of life. These parasites, to which we belong, let the plant collect the energy and build carbohydrates, fats and proteins. Then they simply eat the plant. They perform the last energy-producing cycle themselves. That is they take the hydrogen from these foodstuffs, put it onto TPNH, or a closely-related DPNH, then take electrons from TPNH or DPNH and let these electrons drop to the oxygen level and build ATP with its high-energy phosphates out of the energy thus liberated. We eat plants, or, even do better, we eat the cow, which has eaten the plant.

In this way we can use the energy of the sunshine without carrying the bulky photosynthetic machinery in our bodies. We perform only the last part of this open cycle in which the electrons, coupled to oxygen, yield up their energy. The carbon, which is

left after we have taken off the H-s of carbohydrate, we excrete through our lungs as CO_2, carbonic acid. While the plant thus takes up carbon dioxide and decomposed water, we produce carbon dioxide and water as the final products and use the energy thus liberated to make high energy phosphate bonds, which drive and build us.

Many devoted scientists have contributed to the knowledge I have tried to put before you in a simplified fashion. A great number of laboratories are still at work, trying to clear up the many mysteries still left. These have to be solved to understand this most fundamental process which connects our own lives with the radiation of the sun.

I, myself, with part of my research group, am also working on a special line of this big problem. My problem is what these little electrons do in our cells, how they go from molecule to molecule when their energy is invested into the ATP molecule which has to drive us later. I am led by the hypothesis that, at the origin of life, these excited electrons drove life in a more direct fashion, not only over ATP. I think that what these electrons do is much more intimately connected with the great problems of biology than hitherto believed.

Biochemistry, till now, occupied itself, chiefly, with molecules. I am profoundly convinced that to get to the bottom of things we must descend one dimension lower, to the electron. I do not think that these electrons disappear from the scene when their energy is invested into ATP. I believe that as ATP is formed by investing electronic energy, so electronic energy comes out from ATP again when the ATP drives our cell life. I do not doubt that these little electrons hold the secret to the most important and difficult biological processes. This abstruse and immense field is just opening up. I would like, only, to mention one observation we have made lately. You all know that there are substances capable of producing cancer but nobody has yet explained the way in which these simple chemical substances produce this disturbance.

We have found lately, that all the substances which produce cancer are capable of giving off one electron. So, we have reason to believe that this electron, given off by carcinogens, is actually involved in the production of cancer.

A more detailed study of this phenomenon may lead us to a better understanding of this scourge of mankind.

I have mentioned this result of my studies to show you that if one works on a fundamental line in research, one may be led to unexpected results. I am not a cancer research man; yet, it is possible that my observations will lead to a better understanding of cancer.

If I had, in any way, been limited in my work, I could never have made these observations. Any regimentation of science, any limitation of research, would cut out the unforeseen discoveries which form a considerable part of our present scientific heritage.

We can talk about photosynthesis, carbohydrates, fats, proteins and other foodstuffs with the chemist's abstraction. These words touch upon the most urgent problems of the daily life of mankind. The present great instability of the world is partly due to the fact that mankind is divided into two camps. The life span of the members of the smaller camp is cut short by overeating, while the life span of the great majority is cut short by starvation. So, the solution of the great political problems of our age is intimately linked to the production of foodstuffs, to the methods of agriculture, to agrochemistry and all that which relates to the production of these foodstuffs, the ultimate source of which is the sunshine.

It must be clear to any intelligent mind, that in order to repair our machinery or improve its working, we must know its structure and function. If we want to improve on the production and utilization of foodstuffs we must first penetrate into the secrets of this very complex machinery we call the living organism. A better understanding may lead to a great increase of foodstuff produc-

tion by methods undreamed of today. A great part of the sunshine is still wasted.

Such progress can be made only with an unbiased mind and with complete freedom of thought. Penetrating the secrets of Nature, revealing the structure of its foundation, is what we call basic research. All progress of our age can be traced back to progress in this basic understanding of Nature. One of the most important problems of any administration must be to promote understanding of this basic and fundamental research.

Naturally, money is needed because without instruments and buildings there can be no research. Science does not exist without scientists either, and scientists too need bread and butter to keep alive. But, all this is not the driving force of science, it is not what is driving scientific progress. These are conditions only and not a driving force.

What drove Newton to build his wonderful system of gravitation? What drove Lavoisier to explore burning processes? What drove Einstein? The great progress of science is linked to names such as these, and the names of innumerable other scientists. The only driving force behind all this is human curiosity. Curiosity is its driving force and liberty is the air which it respires.

I am afraid that the present poor teaching methods, used by poorly paid teachers, are more suited to kill than to waken curiosity. This is a most serious problem because the progress of science, the understanding of Nature, is the great challenge of our age, and any nation which falls behind in science will be simply passed by, if not trodden on.

Science has already changed the face of human life and is changing it still today. It will go on changing it at an increased rate, day by day. The future of the nation depends on its science.

This wonderful country, America, which I confess to be my own, has many wonderful minds. If these minds are not allowed to fade away in ignorance but are awakened to the wonderful

challenge of our age, and are given the opportunity to satisfy their curiosity, if the pioneer spirit which has built this country can be shifted into the new spiritual frontiers, then I have no doubt that this country will be second to none. It will be able to answer any challenge and fill that leading position which so many people expect it to fill.

GENETICS

By George W. Beadle

The shift in human cultures from those based largely on hunting, fishing and the collection of wild plant materials for food, clothing and shelter to those based on modern technologies has had profound effects on world ecology. Everyone knows perfectly well that as these technologies increase in sophistication, they come more and more to depend on the sciences that underlie them. Agriculture is no exception, though in some of its branches it has remained largely empirical for longer than have other technologies.

I propose to review some of the recent developments in our understanding of the ways in which living systems transmit directions for development and function from one generation to the next. As we learn more and more about this branch of the life sciences, which we call genetics, it becomes increasingly clear that all creatures communicate biological specifications to their offspring in much the same way, whether they are submicroscopic viruses, bacteria, crop plants, forest trees or man himself.

We have come to recognize genetics as one of the most basic of all the life sciences. It provides us with principles that serve to unify all of biology. Its importance to the agriculture of today is unquestioned. And as the demand increases for food to sustain

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George W. Beadle, who was named Chancellor of the University of Chicago in 1961, has distinguished himself as a scientist and an academic administrator at the California Institute of Technology. His studies of the genetics of the bread mold, Neurospora, led to discoveries that earned Dr. Beadle and his associates—Edward L. Tatum and Joshua Lederberg—the Nobel prize in medicine and physiology in 1958.

exponentially increasing populations of man, its significance to agriculture will surely grow.

The Cell

I should like to begin by reminding you that higher plants and animals characteristically begin development as small almost microscopic single cells, usually egg cells. Man is no exception. The fertilized egg from which we develop is about one-three-hundredth of an inch in diameter—about one-tenth the diameter of the head of a pin. The egg of a horse, a sheep or a pig is not very different in size or appearance. In fact even the egg cell from which a corn plant develops doesn't look so very different. Yet each of these cells obviously "knows" what its potential future is, for gross mistakes do not occur. The egg of a sheep never gives rise to a horse. Clearly the egg cell must contain information that says what it is going to do. A human egg carries specifications for growing into a person—not just any person but a very specific and truly unique one.

It is these specifications about which I wish to speak. I'll begin by posing five questions: First, how are these specifications or directions transmitted from one generation to the next? Second, how are they "written," that is, what is the "language" of heredity? Third, how are they copied or "reprinted," as they must be with each of the many cell divisions that intervene between the egg of one generation and the egg and sperm cells of the next? Fourth, how are these directions "translated" during development? How are they used in the transformation of a tiny spherical egg into an enormously complex plant or animal—one of us, for example? Finally, do errors or changes in the directions ever occur and, if so, what are the results?

Although it is by no means possible to give complete answers to these questions, we can come much closer to doing so than we could a few years ago. Before I go ahead, however, let me restrict my task considerably. The egg cell of an animal is of course com-

plex, despite its small size. It consists of cell membranes, cytoplasm, and a centrally located nucleus.

I shall confine my remarks largely to the nucleus. In so doing I do not in any way wish to leave the impression that I believe the cytoplasm to be unimportant. We know, in fact, that in all cellular forms it is indispensable. It is endowed with highly specific properties and therefore can be said to carry essential hereditary information. This is clear from the experiments of the kind Professor John Moore of Columbia University and several other embryologists have made in which the nucleus of the egg of one species of animal is replaced with that of another. Thus in the two species of frogs, *Rana pipiens* and *Rana sylvatica,* the nucleus of the fertilized egg of one species can be removed or destroyed and replaced by that of the other (Moore, 1958). In whichever direction the switch is made, the egg fails to develop, although after a comparable transfer in which donor and recipient are of the same species development may be quite normal.

The cytoplasm of one species is not compatible with that of the other. This is only one of several lines of evidence indicating that the cytoplasm of the egg has specific properties that cannot be easily modified by the nucleus.

I do not wish to belittle the role of environmental factors. They too are absolutely necessary. In the growth of a human egg cell into a person, for example, a highly specific physical and chemical environment must be maintained, especially in the early stages of development. Raw material of the proper kind must be available at the right times and in the right amounts.

Before and after birth the nervous system is being indelibly impressed with information. Especially after birth the information that is fed into the nervous system in massive amounts plays a large and important role in determining what we are. It includes a large input of cultural inheritance. It is this cultural inheritance, cumulatively increased generation after generation, that makes man unique among all living creatures—that enables

him to evolve religions, art, music, literature, technologies and finally science itself.

The Nucleus

Returning to the nucleus, there is a large body of evidence showing that it contains a very large part of the primary biological information that directs development and function. Mendel demonstrated in his garden peas that this information is particulate. Unfortunately he was far ahead of his time and was quite unable to convince such persons as the German botanist Nägeli that what he found should be taken seriously. As a result his achievements lay unappreciated for a third of a century.

Mendel postulated the units of heredity that we now call genes. He did not know they were in the nucleus of the cell or that they were carried in chromosomes. But he did know that they determine whether pea seeds will be round or wrinkled, whether green or yellow, whether the flowers will be purple or white, and whether the pea plant will be tall or short. He had a remarkable understanding about how genes are transmitted from one generation to the next. Although they have now had to be modified in important ways, Mendel's laws of inheritance still hold as first approximations to the ways of nature in communicating biological specifications from parent to offspring.

We now know that the most basic features of the process of transmission of such biological directions for development are common to viruses, bacteria, algae, protozoa, higher plants, multicellular animals and man. Let me give you an example in man.

About seven out of every ten persons find the chemical substance, phenylthiocarbamide, most unpleasantly bitter. The remaining three find it essentially tasteless. The two classes of people differ in the hereditary specifications. Individuals who taste carry specifications in their cells that somehow say "I can now taste phenylthiocarbamide," whereas those who do not taste this substance carry the alternative form of this message, "I can not taste phenylthiocarbamide." This message, in its two forms, is

one unit of inheritance—a gene. Each fertilized egg, and all the cells descended from it, contain two representatives of this message, one from the mother, contributed by the egg, and one from the father, received from the sperm. There are therefore four possible types of persons with respect to this particular bit of specifications, viz:

From the mother:	From the father:
1. Taste	Taste
2. Taste	Not taste
3. Not taste	Taste
4. Not taste	Not taste

The first is a pure taster. The next two are alike in having one message of each kind. They are "hybrid" for this message. They taste, which is a way of saying the positive taster form of the message is *dominant* to the non-taster form or, *vice versa,* that the negative form is *recessive.*

When sperms or eggs are produced by individuals of these types, each carries one or the other of the two messages, either the maternal or the paternal one. Thus from any pair of parents it is easy to predict statistically the types of offspring, just as Mendel did with his peas.

How many such units or genes are contained in the nucleus of a human egg cell? We do not know with any degree of certainty. But we estimate that it is probably more than 10,000 kinds and less than 1,000,000, each present in duplicate except for sex-linked genes which are for the most part present singly in the male. What do these genes specify? Many characteristics: eye color, hair color, hair form, skin color, and hundreds of other characters by which the species may differ.

You will note, as many biologists did in the early part of the century while the young science of genetics was struggling for recognition, that these are all trivial characters. So they are. And so are most of the characteristics responsible for the uniqueness of

normal individuals of our species. But we now have clear evidence that most genes carry information of vital importance. Thus two genes in man carry the instructions for making the hemoglobin molecules that play such an essential role in carrying oxygen and carbon dioxide. Hemoglobin is *not* trivial.

Soon after the rediscovery of Mendel's classical paper, it was suspected that genes were carried in chromosomes. The clinching proof came in 1915 from the laboratory of the late Thomas Hunt Morgan. His student, Calvin Bridges, showed that errors in transmission of genes are exactly paralleled by errors in chromosome behavior.

The Primary Genetic Material

Chromosomes were known to be made up largely of protein and nucleic acid, the latter of the deoxyribose type. Deoxyribonucleic acid has now become widely know as DNA.

Are the genes protein or DNA—or possibly both? At first their specificities were thought to reside in protein, for chemists knew that proteins are long polymer molecules consisting of linear sequences of amino acid building blocks of some 20 kinds. Since there are obviously almost unlimited possibilities in proprotions and sequences of amino acids, it was easy to believe that the gene was a kind of coded message in protein. DNA, on the other hand, was believed by many to be a rather monotonous polymer built of four kinds of nucleotide units arranged in segments of four that were repeated many fold. In such a structure there appeared to be little opportunity for specificity; therefore DNA was at first not considered a serious candidate for the role of primary genetic material.

A series of investigations on pneumococcal bacteria, beginning in 1928 and leading up to the classical paper of Avery, MacLeod and McCarty (1944), raised serious doubts about the role of proteins as basic stuff of heredity. It was clearly demonstrated that the type of polysaccharide capsule produced by a pneumococcal strain can be altered experimentally by treating it with pure DNA

from another strain. Thus DNA from a type III pneumococcus can cause a type II recipient cell to be transformed permanently into type III, and from then on to produce DNA that specifies type III polysaccharide. The experimental procedure by which this is accomplished is not quite as simple as the above account implies. The results nevertheless suggested that DNA from the donor strain might be entering the recipient and somehow replacing the homologous DNA that specifies the type of polysaccharide in the coat. There were, however, alternative interpretations that continued to be preferred by many geneticists and biochemists.

By 1952 more direct evidence had come from another source. By then the life cycles of certain bacterial viruses—bacteriophages—had been worked out and sufficient genetic work done on them to make it clear that like higher organisms they exhibited particulate inheritance. These viruses, too, have genes.

Bacterial viruses consist largely of protein coats containing cores of DNA. Early electron microscopy suggested that when a bacterial cell is infected by a virus particle, its coat does not enter the host cell. This was most elegantly confirmed by the use of radioactive tracers.

Hershey and Chase (1952) infected bacteria with viruses whose protein coats were labeled with sulfur-35, a radioactive isotope. The labeling was done by growing a crop of viruses on bacteria that in turn had been grown on a medium containing radioactive sulfur in the form of sulphate. After infection, the protein coats were removed from the bacterial hosts by shearing them off in a Waring blendor. Separated by centrifugation, the virus coats and infected host cells could be separately examined for presence of radioactive sulfur. Almost all of the radioactivitiy was found in the virus coats that did not enter host cells. Since proteins contain sulfur while DNA does not, this result suggests that only the DNA enters the host on infection.

Otherwise comparable experiments in which DNA was labeled with radioactive phosphorus-32 led to the same conclusion. In this

case the coats were largely unlabeled after infection had occurred, while the host cells contained most of the radioactivity. Since DNA contains phosphorus—one atom per nucleotide—and protein does not, it is clear that DNA and not much else enters the host cell. Thus, since viruses have genes and only DNA enters the host cell, the viral genes must be DNA.

Actually a small amount of protein does enter with the DNA, but labeling experiments in which the radioactivity entering a host cell in protein or DNA is followed to the next viral generation show that it is the DNA, not the small amount of protein, that is responsible for the transfer of primary genetic information from one generation of viruses to the next.

What about higher organisms—corn plants and man? Are their primary genetic specifications likewise written in DNA?

Although the evidence is not conclusive, it is conservative to assume they are. We therefore proceed on that assumption until evidence to the contrary comes forth.

The Chemistry of DNA

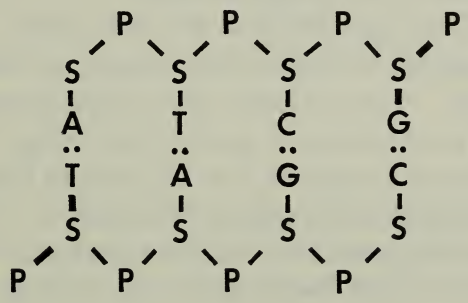
What is the structure of DNA that enables it to carry hereditary information?

A most important step toward answering this question was taken in 1953 by the American biologist, James D. Watson, and the English chemist, Francis H. C. Crick, working together at Cambridge University. Making use of the information then available about DNA—nucleotide composition of DNAs of various sources, X-ray diffraction observations of M. H. F. Wilkins and associates at Kings College London, general knowledge of the structural arrangements of atoms in nucleotide components, etc.—they succeeded in constructing a molecular model of DNA that seems to satisfy all requirements (Watson & Crick, 1953).

It is now generally agreed that the Watson-Crick structure is essentially correct for the native DNAs of a number of organisms.

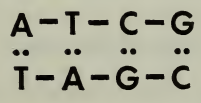
It is also now clear that their achievement is outstanding in modern day biology, for their model goes far in suggesting plausible answers to the questions posed at the beginning of this paper.

According to the Watson-Crick model, DNA consists of a pair of antiparallel polynucleotide chains wound helically around a common axis and cross linked through specific hydrogen bonding between purine and pyrimidine bases. Letting P,S,A,T,C, and G represent phosphate groups, deoxyribose sugars, adenine, thymine, cytosine and guanine, a four unit segment of DNA can be represented in two dimensions as follows:



Paired dots represent hydrogen bonds. Two terminal nucleotides are indicated by heavy lines. These show that the chains run in opposite directions as determined by the orientation of nucleotides.

Omitting the sugar and phosphate components, which are common to all four nucleotides, this can be more simply represented in the following way:



Genetic Information

Genetic information must somehow be determined by sequence of nucleotides. One can think of information being carried either as sequence of nucleotide pairs in the double molecule or as

nucleotide sequences in the two single chain components. In any case, since only A:T and C:G nucleotide pairs are possible in the normal structure, the two single chains obviously carry complementary information. This is of special significance in hypotheses of DNA replication that I shall consider later.

How much DNA is in a single human cell?

It is estimated that the total DNA of the 46 chromosomes of a fertilized human egg contains something like 5,000,000,000 nucleotide pairs. Since the genetic material is carried in duplicate in such a cell, one complete set of information is written in sequence of some 2,500,000,000 such pairs.

Professor Crick has estimated that this amount of DNA is sufficient to encode in duplicate the contents of some 500 large library volumes. This is another way of saying that the genetic specifications for producing a person from an egg cell, given a proper environment, adequate food of the right kind, etc. might be written in English in this number of volumes.

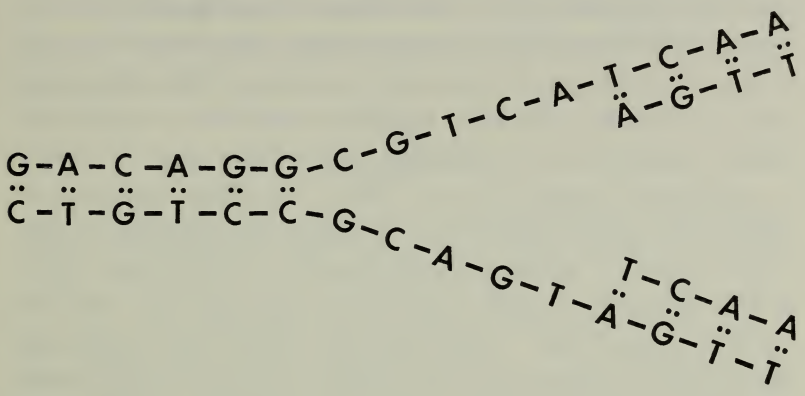
What about the physical size of DNA molecules?

The diameter of the double helix is 20 angstrom units. The base pairs are spaced at 3.4 angstrom intervals. Thus a continuous linear double helix of the 5,000,000,000 base pairs of a single human egg would be somewhat more than five feet in length—only slightly less than the height of an average person. If the strands were packed side by side in a monolayer on the head of a pin one millimeter in diameter, this amount of DNA would cover less than one two-hundredth of the surface.

Gene Replication

The Watson-Crick structure immediately suggested how DNA molecules might reproduce by separation of the double structure into single chains followed by synthesis of new partners against each of the old chains. Since the two originals are complementary, each carries the information necessary to direct the synthesis of its partner. It is presumed that single chains serve as templates

against which free nucleotides are properly ordered by specific hydrogen pairing. Assuming the old chains to remain intact this process can be schematically represented in the manner shown.



Just how the problem of separation of paired chains helically coiled around a common axis is solved is not known. The forces required to "untwist" the double helix would not be great if it were to rotate around its axis in the manner of an automobile speedometer cable.

What is the evidence, if any, that DNA replication in fact occurs in this way?

There are two kinds of experiments that bear directly on this matter. One of these depends on labeling old and new chains so that they can be distinguished. This can and has been done with radioactive phosphorus-32, but there are difficulties in this method that have not been overcome in an entirely satisfactory manner. A second method of labeling involves the use of the stable heavy isotope of nitrogen, N^{15}. Bacteria grown on a culture medium containing only N^{15} eventually become fully labeled with this isotope. Since there are eight nitrogen atoms per pair of nucleotides of molecular weight approximately 700, replacing all N^{14} with N^{15} increases the weight slightly more than one per cent.

Since the size does not change, the density increases by a corresponding amount. "Heavy" DNA molecules can be separated from the light variety in an analytical ultracentrifuge.

The method for doing this was first developed by Meselson, Stahl and Vinograd (1957) and consists of centrifuging DNA in a cesium chloride solution of proper density. The cesium chloride molecules are thrown down in a high centrifugal field. But, being small, they diffuse sufficiently rapidly to establish an equilibrium density gradient in the centrifuge cell. If the range of density so established includes that of DNA, the DNA molecules in solution will form a band at a level exactly corresponding to their buoyant density. Those at the centripedal end of the cell "sink" to their proper level while those at the centrifugal end "float" to the same density level. Being large they diffuse only slowly and hence form a narrow band, the position of which is easily established by means of an ultroviolet optical system. A mixture of N^{15} and N^{14} DNA molecules form two cleanly separated bands.

This method makes possible the following elegant experiment first carried out by Meselson and Stahl (1958). Bacteria are grown on N^{15} medium until equilibrated, that is, until they become uniformly heavy. The bacteria are then transferred to a medium containing only nitrogen of atomic weight 14. After one cell generation, as determined by doubling of the total population, all DNA molecules should have one heavy old chain and one light new complement. That is, they should be "hybrid" and hence intermediate in density between heavy and light DNA. They are. In a second round of replication—which doubles the population again—heavy and light chains should separate and each then direct synthesis of a light complement. Hence, half the DNA molecules after exactly two cell divisions should be hybrid and half light. Again they are.

If a population of hybrid DNA molecules—present after one replication of heavy molecules in an N^{14} medium—are heated under the right conditions, molecules about half the molecular

weight are found and they are light and heavy in equal numbers. The evidence is fairly convincing that these are single chains of DNA.

This experiment does not prove that the Watson-Crick hypothesis of DNA replication is correct but it strongly suggests it. A perverse nature might have devised another way of giving the observed result.

DNA Replication

An even more dramatic way of investigating the mechanism of DNA replication is that devised by Arthur Kornberg and his coworkers (1959). In a suitable buffer solution containing magnesium ions, the four nucleotides of DNA as triphosphates, and a DNA polymerizing enzyme, DNA is rapidly synthesized if primer DNA molecules are added. Single stranded DNA, obtained by heating native DNA, is much more effective as a primer than is carefully prepared native material. Something like a ten- to twenty-fold increase in DNA over that added as primer has been obtained.

That the primer is copied is suggested by the fact that the base composition of the product is like that of primer. DNAs from different sources may have quite different ratios of A:T to C:G base pairs, and it is therefore possible to determine whether the ratios of various primers are reproduced in the newly synthesized DNA.

Without primer, DNA is spontaneously synthesized in the Kornberg system after a lag of two to four hours. But unlike natural DNA, this spontaneously synthesized material contains only A:T base pairs. If this A:T copolymer is now used as a primer in a fresh system, more A:T polymer is formed without a lag period. In this process C and G nucleotides are excluded although present in abundance in the system. Here too it appears that the primer is copied as the hypothesis predicts.

Again the agreement between hypothesis and the facts observed in the Kornberg *in vivo* synthesis of DNA does not prove the hypothesis beyond all doubt. But it does enormously strengthen the case.

The Translation of Genetic Information

How is information in the form of DNA used in the development of a complex organism?

This is clearly a question of the most fundamental importance to biology. At the same time it is an enormously difficult one and we are a long way from knowing the complete answer.

All living systems contains DNA (or in some viruses a related form of nucleic acid, ribonucleic acid called RNA) and protein. In cellular forms the proteins are of many kinds. We know that many genes, perhaps all, are somehow concerned with synthesis of specific proteins. Many of these serve as organic catalysts—enzymes—or as components of these catalysts. Enzymes accelerate vital reactions that would otherwise proceed at rates too low to sustain life. In cellular organisms there are thousands of kinds of enzymes, each owing its specificity to a particular protein. We can therefore narrow the problem of gene action, at least in some cases, to that of protein synthesis.

An hypothesis at present widely used as a working basis visualizes the process of protein synthesis in the following way: For each protein potentially capable of being formed, there is in the nucleus a specific segment of DNA that carries the information by which the twenty kinds of amino acid subunits in that protein are properly ordered during its synthesis.

Take human hemoglobin as an example. Each molecule of this vital oxygen-carrying red protein consists of four protein chains and an equal number of heme groups each containing an atom of iron. The protein chains are of two kinds, called alpha and beta chains. There are two of each, the members of a pair being

identical and each made up of about 150 amino acid units arranged in a precisely determined sequence. For each of the two kinds of protein chains, there is presumed—with some evidence—to be a gene consisting of a segment of DNA. This segment is, by the definition I shall use, a gene. It may be something of the order of 1000 nucleotide pairs in length.

How is the information in the gene for the alpha hemoglobin chain used?

The hypothesis assumes the following sequence of events: From the gene in the nucleus, information is transferred to RNA, possibly by the DNA somehow acting as template in the ordering of the four kinds of ribose nucleotides in RNA. This informational RNA then moves from the nucleus to the cytoplasm of the young red blood cell—before it loses its nucleus. There, or possibly in the nucleus before the migration, it is incorporated into microsomes. These are submicroscopic bodies made of structural protein and structural RNA. Once in microsomes, informational RNA molecules serve as templates against which amino acids are arranged in proper order to make alpha hemoglobin protein chains.

Prior to this the amino acids are activated and attached to small carrier segments of RNA, each specific for its own amino acid. Thus for each of the twenty amino acids there is a corresponding carrier RNA segment. Carrier RNA molecules are somehow coded to specific sites on the informational RNA in the microsome. In this way each amino acid is carried to its proper position on the template. They are then joined through peptide linkages to form alpha chains and are released from the template and ribosome. Whether association of alpha and beta chains with their hemes, and with each other, occurs in the microsome or outside is not yet known.

Not all proteins for which genetic directions are available in the nucleus are synthesized in any one cell. There are ingenious

control mechanisms in operation which determine whether the information in a given gene will be used and, if so, when and for how long. In some cases it is clear that the presence or absence of substrate determines whether a given enzyme will be synthesized. Thus enzymes are not made in quantity when there are no subtrates on which they can work.

Through studies on such feedback and other control mechanisms we are beginning to understand how it is that cells with identical genetic information in their nuclei may do quite different things. It depends on their environmental context or on their previous history.

There are now known many instances in a variety of organisms in which specific protein variation is known to be related to particular genes. I have already referred to hemoglobin as protein in man. Similar situations are known in viruses, bacteria, algae, fungi, insects, higher plants, and mammals. (Beadle, 1960).

The hemoglobin case in man is interesting in that it is known that gene changes may result in substitution of a single amino acid in a chain of 150 units. Thus sickle cell hemoglobin, synthesized under the direction of a modified form of the gene in control of the beta protein chain, differs from normal hemoglobin in that a single glutamic acid unit in the chain is replaced by a valine. In the presence of a third form of this same gene the same glutamic acid unit is replaced by the amino acid lysine. In all about a dozen modifications of human hemoglobin are known, many of them investigated genetically.

Another example in man is found in the genetic disease galactosemia. Here a specific enzyme, galactose-1 phosphate uridyl transferase, essential for the conversion of galactose into a usable form, is partially or wholly inactive. The enzyme defect is referable to a gene change. Galactosemia is a serious disease that leads to serious impairment of the central nervous system and consequent mental defect. Diagnosis is possible immediately

after birth. Knowledge of the nature of the biochemical defect makes possible circumvention of the disease through use of a galactose-free diet.

Obviously such circumvention does not correct the basic genetic defect. Therefore if "cured" galactosemics reproduce, the frequency of the disease will be expected to increase in subsequent generations. Fortunately the increase in frequency of the defective form of the gene is very slow. Nevertheless society may eventually find it necessary to face a eugenic problem that will increase in importance as modern medicine achieves greater success in circumventing this and other genetic diseases.

In bacteria, fungi and other microorganisms correlations between gene change and specific protein modification are much easier to detect than they are in man with his several obvious disadvantages for biochemical and genetic investigations. As a result there are dozens of examples known in such forms as *Escherichia, Salmonella* and *Neurospora.*

Gene Mutation

In terms of DNA structure gene mutation is believed to be the result of alterations in nucleotide sequence. Many mutations are thought to be the result of errors in DNA replication. In their original paper on DNA structure, Watson and Crick pointed out that if at the precise moment of partner selection a purine or pyrimidine were to exist in an improbable tautomeric form, it might form hydrogen bonds with a "wrong" nucleotide. At the next round of replication such a "wrong" nucleotide would direct that its own complimentary nucleotide be inserted in the paired chain. Thus one of the two daughter DNA molecules would differ from its sister in one nucleotide pair. Its informational content would be modified accordingly.

Experimentally, mutations can be increased in frequency in a

number of ways. High energy radiations—ultraviolet and X-rays —are effective in doing this. Transmutation of artificially incorporated radioactive isotopes such as phosphorous-32 is known to produce mutations. Since radioactive isotopes occur naturally in low frequencies, they are no doubt responsible for a small fraction of spontaneously produced mutations.

Many chemical substances are known to be mutagenic. Perhaps the one best known from the standpoint of mechanism of action is nitrous acid. This specifically oxidizes amino groups. In this way it may chemically change a natural DNA pyrimidine or purine in such a way that its hydrogen bonding specificity is modified.

The mutagenic properties of nitrous acid have been especially well studied in tobacco mosiac virus, an RNA virus. Here the natural pyrimidine, cytosine is converted to uracil, which is also a naturally occurring pyrimidine in RNA. Adenine is oxidized to hypoxathine, the latter not a normal constituent of RNA. In a similar way guanine is changed to xanthine. It appears that the oxidation of any one of 3000 of the 6000 nucleotides in a tobacco mosaic virus particle results in an inactivating mutation.

The purine and pyrimidine analogues, 2-aminopurine and 5-bromouracil, produce mutations, premumably by being incorporated in replicating DNA in place of their natural counterparts and thereby leading to errors in complement selection. Since these analogues presumably replace their natural counterparts, purine for purine and pyrimidine for pyrimidine, it might be expected that they would be effective in reversing the mutations they induce. In a special class of bacterial virus mutants, investigated by Ernst Freese, this seems to be the case, whereas other classes of mutants, for example those induced by proflavine, are not so reversed by base analogues. It is presumed that the latter brings about replacement of purine by pyrimidine and vice versa. Investigations of this kind offer the hope of giving us a deeper understanding of the mutation process than we now have.

Fine Structure of Genes

If, as I have suggested, genes as functional genetic units of DNA are hundreds or thousands of base pairs long, it ought to be possible experimentally to demonstrate multiple mutational sites within a single such unit. Furthermore these should be arranged linearly within a unit. So called fine-structure studies on genes of bacterial viruses, bacteria, fungi and other organisms, show that this is indeed the case (Beadle, 1960).

Clearly the unit of mutation is much smaller than the unit of function, for such sites within a functional unit undergo recombination in much the same way as do separate genes within a chromosome. On the basis of frequencies of such recombination it is possible to construct intragenic maps of mutational sites and thus to show that they are linearly arranged. The shortest distances that can be measured by such recombination are not far from those that are calculated to exist between adjacent nucleotides in DNA.

The Coding Problem

How are sequences of nucleotides in DNA related to amino acid sequences in proteins?

DNA and protein can be thought of as four- and twenty-symbol systems respectively. If they are both equivalent to simple linear codes, it is clear that it requires at least three nucleotides to specify an amino acid, for the maximum of "two-letter words" is only sixteen. Several possible coding systems have been investigated but so far the problem has not been solved (Levinthal, 1959). Perhaps it will not be until nucleotide sequences in a gene can be compared with amino acid sequences in the protein specified by that gene.

As you know, complete amino acid sequences have been worked out for only a very few proteins and unfortunately these are from organisms that are far from ideal for genetic study. There are now investigations under way in many laboratories designed to

correlate genetic fine structure with protein fine structure. Virus proteins and bacterial enzymes such as alkaline phosphatase and tryptophane synthetase are among the systems that look especially favorable for such combined genetic and chemical studies.

Evolution

Presumably DNA replication bears no immediate relation to its informational content. Biologically useless DNA is replicated just as faithfully as that playing a vital role. Presumably, too, the mutation process is a random affair. If so, meaningful DNA sequences are much more likely to be made less useful through random mutation than they are to be converted into sequences more useful to the organism of which they are a part. In this sense they may be likened to random typographical errors in a useful message. Direct experiment verifies this expectation; most mutations are unfavorable in the context in which they occur.

If mutations are mostly unfavorable but are as faithfully reproduced as are their normal counterparts, why do they not accumulate with successive rounds of replication? A typist would accumulate errors in a message if she were to type copies in succession, each from the previous typing, in a purely mechanical way without proofreading or correcting errors.

The answer is that biologically unfavorable mutations specify organisms with reduced reproductive fitness. The errors in their DNA are not corrected, but the lines of descent carrying such errors are statistically bred out of existence over a shorter or longer number of generations. It depends on the degree of reduction in reproductive fitness. We call this natural selection.

Positive evolutionary progress depends on rare favorable mutations that increase reproductive fitness. They gradually replace the ancestral types from which they arose. It should be emphasized, however, that mutant forms of a gene cannot be defined as favorable or unfavorable in any absolute sense. Just as a

given word is "good" or "bad" according to the context in which it appears, so a given gene will be favorable or unfavorable depending on both its genetic context and the environmental context in which the organism that carries it finds itself.

A mutant conferring resistance to DDT may be highly advantageous to a house fly in an environment in which DDT is present. But it does not follow that the same gene will be favorable in a DDT-free environmental context. Circumstantial evidence in fact suggests that it will be unfavorable.

Modern concepts of organic evolution hold that all living systems have evolved gradually, mutation by mutation, from preexisting organisms. Sometimes as in parasites, it is advantageous to become simpler and to count increasingly on the host for raw materials, a proper environment and protection against enemies. But from the beginning, the over all trend in many lines such as our own has evidently been toward greater complexity.

Speculation as to how organic evolution began in the first place leads to the conclusion that there is no clear qualitative break in the sequence of events that spans the advent of life on earth. This is only another way of saying we cannot clearly distinguish between living and non-living systems. No matter what system one contemplates, it is possible to imagine a closely related one, only slightly simpler, that could have given rise to the former by a single mutation-like step.

The simplest living systems we know today are viruses. Tobacco mosaic virus particles are submicroscopic rods about 800 Å units in length. They consist of a cylindrical protein coat and an RNA core, the latter being made up of about 6000 nucleotides. In a living tobacco cell infected with a particle of this virus more viruses are synthesized. The bacterial virus ØX174 contains about the same amount of nucleic acid. Its units are almost spherical. They too have a protein coat and a nucleic acid core, but their nucleic acid is DNA, not RNA.

Some people say such viruses are not living—that they cannot carry on metabolism, synthesize their component parts, or do any of several other things that living organisms do. But they do reproduce their kind, given a proper environment. And they are mutable, and hence capable of organic evolution. Whether one calls them living or not depends on one's definition of life and that in the end must be purely arbitrary.

Let us imagine that virus-like systems were among the earliest "organisms" to evolve on earth. They were not like present day viruses, for there were no living cells from which they could derive their parts—nucleotides, amino acids, and perhaps a few enzymes. We can reasonably believe that at that stage of the earth's history there were myriads of spontaneously formed organic molecules around, including nucleotides, amino acids, and proteins. These were the building blocks of the postulated virus-like creatures. After all we now have abundant experimental evidence that a variety of organic molecules are formed spontaneously under conditions assumed to have been characteristic of parts of the earth's crust a few thousand billion years ago (Miller, 1957).

If the building blocks of the postulated virus-like systems were around, would they not have interacted, again spontaneously, to form nucleic acids, proteins, and the like?

Nucleotides interact in the Kornberg system to form DNA capable of replication. Since the role of man in this experiment is merely that of making the conditions more favorably for this particular reaction—that is, increasing its probability—would it not have occurred in his absence when the conditions became right?

As every organic chemist knows, organic molecules are formed through the interaction of inorganic molecules—again when the conditions are right. And as every inorganic chemist knows elements interact to produce inorganic molecules. Nuclear physicists tell us that elements themselves evolve from simpler elements as

a result of processes that are both natural and inevitable, given the appropriate circumstances.

Thus it is clear that the sequence: hydrogen - helium - beryllium-8 - carbon - oxygen - other elements - water - other inorganic molecules - simple organic molecules - more complex organic molecules like nucleotides, amino acids, and small proteins - nucleic acids capable of replication - nucleic acids protected by protein coats - virus-like systems with protein coats serving catalytic functions - multigenic but subcellular organisms - simple cellular systems like bacteria - autonomous cellular forms like algae - protozoa - multicellular plants and animals and, in our line of descent, man himself - is a natural one that could have arisen by steps no one of which need have been larger than the individual mutational steps we know in today's living systems.

In the beginning there was a universe of hydrogen. How and when it was created—or whether it is and always has been in a steady state of continuous creation—science knows very little. In whatever way the universe began, there must have been built into it in the very beginning the potentiality of essentially unlimited orderly evolution.

Man in Control of Evolution

It is obvious that we—and we alone among all the organisms on earth—have a genetic endowment that has made possible the evolution of a culture that depends on the progressive build-up of knowledge and understanding and its transmission from generation to generation in ways that both transcend and supplement our purely biological inheritance. The science component of our culture has now developed to a level that permits us to take over the deliberate direction of the evolution of the plants we cultivate and the animals we domesticate. Our success in actually doing this is in large part responsible for the remarkable achievements

of modern agriculture. I wish to emphasize, however, that we have so far done only a tiny bit of what is potentially possible.

The knowledge by which we control the evolutionary futures of the plants and animals that serve our needs is just as applicable to the direction of our own evolutionary futures. We are capable of supplementing and in some respects replacing the forces that have got us where we are. But just as society as a whole—not scientists alone—must decide how we will use our knowledge and power in other respects, so is the decision as to whether or not we plan our own evolution in any systematic way—one that society as a whole must make.

NUTRITION—THE CHEMISTRY OF LIFE

By Jean Mayer

It is particularly fitting that the nutritional aspects of the chemistry of life should be emphasized in a lecture given under the auspices of the United States Department of Agriculture. Few institutions have contributed as much to any one science.

We often hear that sciences really have their roots in Western Europe, that much of the basic thinking is still done there, and that the United States has only developed applications for these sciences.

Nutrition is a case where this is emphatically not so. One can say that the big share of thinking in nutrition in this century has been done in the United States, largely in agricultural stations, schools of agriculture, and Land-Grant colleges. Much of the early work in public health nutrition and in the social aspects of nutrition emanated from this Department.

For example, the first complete food composition table was experimentally compiled by Atwater at the Connecticut Agricultural Experiment Station. Then T. B. Osborne, of the Connecticut Station, worked closely with Lafayette B. Mendel of Yale in making the first experiments on the biological value of proteins. Such names as Steenbock, Elvehjem, Maynard, and Brody testify to the vigor of nutritional research in schools of agriculture.

The U. S. Department of Agriculture took the lead in applying this knowledge in the depression years. Hazel Stiebeling, now Director of the Institute of Home Economics, was among

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Jean Mayer of the Harvard School of Public Health has gained wide recognition for his research on the biochemistry of obesity and appetite control. Dr. Mayer, a native of France, earned his doctorate at Yale in 1948.

those who pointed out the nutritional implications of the great depression as a basis for rational action.

In nutrition as in other fields, the present is the child of the past. The history goes back to the Stone Age. This period came to an end in the second half of the 18th century when Lavoisier introduced calorimetric concepts and measurement.

The next period in the development of our knowledge of nutrition extended over the 19th century. It was devoted to studies of caloric and nitrogen balance.

The third period—extending over the first four decades of the 20th century—was marked by the discovery of trace elements, vitamins, and essential amino acids and the intensive study of deficiency diseases.

The fourth period began about the time World War II ended. It has been characterized by studies of the role of nutrition in degenerative diseases and by the recognition that what is in the diet can be almost as important as what is missing from it.

In the long period stretching back to the cave age, our ancestors learned by trial and error, very often mortal errors, to identify those plants and animals that could be used as foods and would not cause immediate disease. They learned where and how these plants and animals could be grown. And they developed cooking. When we sit down at a meal today we apply not only modern knowledge but a tradition of human endeavor that stretches back for many centuries.

The idea that food does more than assuage hunger is very old. Certain beliefs such as the idea that eating the heart or flesh of brave or strong animals would confer the same virtues to the warriors of the tribe appeared with the dawn of human history. Food taboos also appeared during the Stone Age. Some of them may have had something to do with the wholesomeness of food. For example, the Biblical prohibition of pork may have been based on the recognition that swine are frequently infested with Trichinelli. Most of the edicts seem to have no basis in fact.

Examples are the Egyptian prohibition of beef or chicken for kings and the Biblical edict against hare.

Closely allied with the taboos and superstitious beliefs was the search for foodstuffs that also served as remedies. Many herbs and parts of plants or animals were prescribed because of their shape, their color, or some other property unrelated to any demonstrable pharmacologic effect. Among early examples of justifiable prescriptions for deficiency diseases were the recommendations of the "Ebers Papyrus" (1600 B.C.), of Hippocrates in the 5th century B.C., of medieval writers who advocated liver for eye diseases and night blindness, and of Cartier's Indians who used evergreen needle infusions for scurvy.

Hippocrates, the founder of scientific medicine, paid considerable attention to nutrition in his writings. Many of his opinions are difficult to justify—for example, his belief that beef is more difficult to digest than pork or that fish should be roasted rather than boiled for feverish patients. But his abhorrence of extremes —both abstemiousness and diet restriction and of excessive intake without corresponding physical labor—is still good nutritional advice.

The great turning point in the history of biology and medicine was the investigation of combustion by Lavoisier (1743 to 1794). He introduced measurements in biologic and chemical studies and in these experiments laid the basis for our understanding of calorie expenditures and requirements.

Specifically, Lavoisier showed that oxygen was used to burn foods. The smallest amount of oxygen was used when the individual was at rest at a comfortable temperature several hours after he had eaten. This is very close to what we call the condition of basal metabolism. The figures Lavoisier obtained for this oxygen consumption—with primitive equipment—were close to the figures we still recognize as normal.

Furthermore, Lavoisier showed that an individual increased oxygen consumption when he took food, became cold, or in-

creased muscular activity. In fact, this could double or even triple the basal energy requirement.

A great deal of work since Lavoisier's studies has been to get more specific figures for the cost of the various activities and the cost of keeping body temperature constant under various climatic conditions. As an upshot of this work we can ascribe a figure, in terms of calories, to all human needs. We can calulate with a great deal of accuracy how many calories this or that particular man, woman, or child will require to live a particular type of life.

A third important phase took place in the 19th century with the discovery that all forms of calories are not equivalent. Protein plays a very special role. Carbohydrates can replace fat and vice versa from the point of view of calories available to the body. The same replacement can not be made for protein. There is a minimum protein requirement.

Vauquelin, Magendie, Prout, Mulder, and especially, Von Liebig (1803 to 1873) developed concepts and methods of analyses permitting the establishment of food composition tables. These are still the essential tools of the nutritionists. Any re-assessment of the value of a diet and dietary recommendations must be translated in terms of food to be of practical use.

Still, nutritional knowledge was very simple when Atwater began his work. It appeared that the nutritional requirements of an individual could be met by calculating his need for calories and making sure the calories contained enough protein.

The revolution in the thinking on nutrition which took place between 1905 and 1910 has rarely been paralleled in the history of medical science. It was like the Pasteurian revolution.

The turning point came with the concept of essential nutrients. This was tantamount to recognizing that the organism is a good but not a perfect chemist. It can synthesize thousands of complicated molecules. It cannot synthesize a number of structures—vitamins and essential amino and fatty acids.

Hopkins showed tryptophan to be such an indispensible nutrient. Osborne and Mendel discovered that zein, the chief corn protein, is low in two amino acids—tryptophan and lysine. Adding them to corn proteins improves the biological value.

McCollum and Davis used semi-synthetic diets in experiments with rats to demonstrate requirements for two unidentified factors in addition to long-known nutrients. They called the unknowns "fat-soluble A" and "water-soluble B."

The clinicians—Linde, Trousseau, and Eijkman—were the first to implicate nutrition in scurvy, rickets, and beriberi. However, it is to the chemists—Hopkins, Mendel, McCollum and others—that we owe the concept of deficiency diseases as clinical entities.

The work led on to the recognition that there are about 10 essential amino acids for man. Under various circumstances this one or that one may be a limiting factor.

As the chemists identified the compounds and determined the amounts needed to make the fat soluble vitamins—A, D, E, K, and the water soluble vitamins—various B vitamins and C—it became possible to describe man's need for food with great accuracy and in much detail.

The new knowledge came at a time when it could be translated very easily into social responsibility. It was the period of the great depression not only in the United States but throughout Western Europe. Many governments had a great many people to be fed. Most of these people were unemployed. For this and other reasons they were unable to fend for themeslves. The new body of knowledge of nutrition made it possible to plan in some detail how they should be fed.

The first meeting of the League of Nations committee on nutrition took place in the early 1930's. It marked the first time that an international agency took social action before the individual governments had come to grips with the problem.

A second League of Nations committee brought together nutritionists, physiologists, economists, and agriculturalists. It prepared a report that has been called the marriage contract of health and agriculture. For the first time the new knowledge of nutritional requirements was translated into needs for certain foods and recommendations to government on food planning, nutrition education, and the machinery by which these needs would be met.

Research on nutritional requirements continued actively during the 1930's. Pyridoxine, niacin, and other B vitamins were isolated.

The intellectual climate of the Western world was ready in 1944 to implement the war aim, freedom from want. You will recall this was one of the four freedoms proclaimed by the Allies.

The Food and Agriculture Organization of the United Nations was created at a conference in Hot Springs, Va. It was the first of a number of organizations dedicated to the elevation of nutritional levels and the application of nutritional knowledge throughout the world. UNICEF, UNESCO, and WHO—all have programs related to nutrition.

I would like to emphasize at this point that it is not enough to know what nutritional requirements are. Malnutrition is increasing in many parts of the world.

For instance, the spread of starch roots such as cassava has increased protein malnutrition throughout Africa. The extension of modern rice milling practices that remove the outer husk has resulted in more beriberi, the vitamin B_1 deficiency syndrome, in countries where rice is the main food.

We have at least one example in the United States where economic problems, as such, are not the paramount obstacle to good nutrition. The experience with fluoride shows that it is not enough to know a nutrient is missing and can be furnished, in this case, by fluoridation of the water supply. You still have to convince the inhabitants of the various towns that this is a good thing.

And you encounter tremendous opposition from certain sects, food faddists, and other groups.

There are many unanswered questions about deficiency diseases. For instance, what amino acids specifically are missing in the diet of this or that population? What are the specific effects of amino acid deficiencies as compared to protein deficiency as a whole? These and other questions must be answered. But if we consider the progress that has been made in this research we can guess that advances in this area will be less dramatic than in the past.

We have reached another turning point in nutrition research. The renewed interest in *undernutrition* during World War II led directly to an interest in *overnutrition.*

Observers in Leningrad were struck by the drastic drop in the number of patients hospitalized with cardiovascular illness during the famine when the city was besieged. Afterwards, the "re-feeding" period was accompanied by an upsurge in these illnesses.

This and other reports led us to think that perhaps the quantity of diet and the replacements of one food by another may have important effects on degenerative diseases, particularly on heart disease. After all, coronary thrombosis and other cardiovascular diseases kill the majority of Americans and people of Western Europe.

The present trend in nutrition research is in basic studies. The goal is to identify the causes of obesity, which contributes to many degenerative diseases, and those nutritional factors that cause or are related to the development of heart disease.

We are back where our predecessors were in 1910. The problem, then, was not to devise enrichment programs or find vitamin preparations small children would accept. It was to identify nutritional requirements.

Similarly, today, we are just trying to learn the factors, which have to do with the development of these diseases. Spectacular

advertisements notwithstanding, no miracle cures are in immediate prospect.

Ancel Keys, among others, has observed that there seems to be an inverse relationship between the total amount of fat consumed in a country and freedom from heart disease. Or more specifically, the more fat that people consume, the more they die from heart disease.

The general relationship seems to hold true but this simplified presentation raises all sorts of difficulties. Keys, himself, recognizes them. For example, when fat consumption went up, carbohydrates usually went down. Why assume that carbohydrates are not important. Similarly, when fat consumption went up so did a great many other things—the number of automobiles and telephones in the country.

The interpretation of the data, though suggestive, certainly didn't lead to a simple prescription for the prevention of heart disease. The experimental work, however, confirmed that when the diet was high in certain fats, particularly the hard fats, the cholesterol level in blood was high.

I may add that, while the relationship between high fat diets and cholesterol is well established, the correlation between a high cholesterol level and the prevalence of coronary catastrophes is not so clearly demonstrated. The presumptive evidence is in its favor.

It was quickly shown, however, that not all oils were equivalent in lowering serum cholesterol. Olive oil is relatively poor for this purpose, corn oil, cottonseed oil, and the fish liver oils are very good. There is some indication that factors other than the fatty acids in the oils may affect blood cholesterol.

Recently, it has been shown that factors other than fat may effect cholesterol level. For instance, the replacement of starch by sucrose in the diet seems to lead to higher blood cholesterol levels. Certain derivatives of nucleic acids also have an effect.

All of these are preliminary findings. We really don't know what factors in nutrition influence blood cholesterol. More important, we know very little of the interaction between these factors and such things as decreased physical activity and stress.

We may make some tentative judgments to the effect that it is probably not wise to derive too many of our calories from fat. And it is not wise to derive too many of our calories from hard fats. This is about as far as we can go. The needs for research in this area are tremendous.

Similarly, the needs for research in obesity are considerable. To hear many laymen, and for that matter, many clinicians talk, you would think we know all there is to be known about obesity.

You would hear, first of all, that we know how to diagnose it. All we need to do is look at a person's height and weight and then check in a table to see if he is too heavy.

You would think we know exactly what the relationship of obesity is to disease. It is bad. In all cases it increases the incidence of disease. You would think that we know very well how to cure it. The cause of obesity is overeating. You exercise some self-control, eat less, and there is no problem.

Actually, this is an area where—to paraphrase Archibald MacLeish—we know all the answers; it is the questions we do not know.

First of all, we don't know too well how to diagnose obesity. Certainly it is not weight, as such, that we are interested in reducing. After all, the forward lines of every American football team are made up of individuals who, by life insurance tables, are too heavy. In fact, many of their predecessors had trouble getting into the armed forces during the war because of their excessive weight. We are not, however, concerned with excessive weight if it is muscle. At least we don't think we are. We are concerned with excess weight if the excess is fat.

Our interest is in finding a practical method for estimating body

fat. Estimates, at present, are made by the old Archimedes method of immerging the object, the patient, in this instance, and determining his density.

We also use the fact that half of the body fat is deposited under the skin and can be measured by pinching the individual, preferably with a caliper if you want to be scientific. Tables are being developed which will permit a calculation of total body fat from selected skinfold thickness data.

We need better and quicker criteria. From the practical viewpoint, however, I think that one observation alone does well. If you look fat to yourself you probably are. This is a better criteria of obesity than height-weight tables.

Now the relationship to disease is something we need to know more about. It is probably much more complicated than we first thought. It is not likely that a strict casual relationship is always at stake.

Quite rightly, much has been said about the high rate of mortality in obese people from diseases of the heart, kidneys, and liver. Very little has been made of the fact that these obese individuals have a lower mortality from suicide and tuberculosis. Certainly, nobody thinks of a strict causal relationship here. People don't think an obese individual is less likely to commit suicide because he doesn't have the energy to drag himself through the window or can't find a strong enough rope.

More likely, some of the psychological factors which make for obesity also make for a decreased tendency to suicide. We don't know. Similarly, obese individuals are less likely to become infected with tuberculosis in important ratio.

The observations open all sorts of interesting possibilities in the relationship between immunities and general metabolism. On the other hand, it may well be that some of the relationship between obesity and heart disease is association rather than causation. For example, lack of physical activity may make for both increased fat content and increased heart disease.

Experimental evidence suggests that a disturbance in carbohydrate metabolism may lead to both obesity and an insulin-resistant diabetes often seen in people of middle age. It may thus well be that the same basic disturbance leads to increased appetite and diabetes.

This brings me to the last and a very important aspect of this research. We do not know enough about the normal regulation of appetite. Until we understand that, it will be very difficult to reduce an appetite which is pathologically elevated.

Lack of will power—so often blamed—seems somewhat oversimplified when you think that the century has been peculiarly that of the obese dictator. No one has accused Stalin, Goehring, Tito, Franco, or Peron of having no will power. So there is obviously more to it than that.

The great failure of weight control programs just points to the fact that we don't really know how to start.

In recent years, we have been getting some clues as to what regulates normal appetites. We have shown that there are centers in certain parts of the mid-brain, the hypothalamus, which have to do with food and water intake, and, in some cases, with spontaneous physical activity. Destruction of some of these centers causes an animal to overeat and become obese. This destruction can be done by surgery, burning certain areas, or by injecting with a chemical that will hit the centers specifically. Destruction of a center in the more lateral part of the mid-brain causes the animals to stop eating. Other centers have to do with thirst.

A great deal of our experimental work at Harvard seeks to define the interrelationship of thirst and hunger centers. We have been able to show that those same centers in mid-brain direct and regulate the gastric hunger contractions, the ryhthmic movements of the stomach that go with the feeling of hunger in many individuals. We are trying to track the upward projections in those centers. In other words, how do hypothalamic phenomena get translated into conscious sensations at the cortical level?

This is not simply a physiological problem. It is obviously a psychological problem. New conditioning and behavior techniques allow us to analyze in some detail the modifications of eating and drinking patterns when this or that center is destroyed.

For instance, we can teach animals to feed themselves by pressing buttons and receiving pellets of food. They feed themselves intragastrically by pressing buttons and receiving food in a stomach fistula. They feed themselves intravenously in the same way. They even press buttons that directly stimulate centers in the mid-brain having to do with food.

Now any complicated mechanism can go wrong in a great many different ways. We have induced obesity of eight or ten types in experimental animals. These types differ considerably. They have only this in common—the animal eats more than it expends and therefore becomes obese. The things that increase food intake have no more in common than those that decrease it. For instance, your food intake may decrease because of bad news, unappetizing food, a cold, a blow on the head, or other causes that have little to do with one another.

We have induced obesity in experimental animals through various types of genetic transmissions—recessive, dominant, sex linked, and others—through the action of certain chemicals and drugs, through surgery, and finally through feeding unbalanced diets extremely high in fat and immobilizing the animal. Farmers have known for a long time that immobilizing an animal can make it quite fat. People tend to forget this when they minimize the importance of exercise in weight control.

I have gone into some detail to show that the end point of our research is not a miracle prescription available at any drug store or supermarket that will enable you to replace food with a liquid preparation and get thin or to replace butter by a particular brand of margarine and avoid heart disease.

Instead, we are really groping to discover those mechanisms and

basic factors that are involved in the great killers of mankind, the degenerative diseases. We need more of this basic research.

If I may end this talk on a note of pride in my own science, I would like to point out that nutrition has this very satisfying aspect. There is probably no other branch of research where the basic findings can be translated so quickly and on such a large scale to the improvement and preservation of human welfare.

A nutritionist is someone who can go from the test tube where he studied the mode of action of enzymes to the organization of a nutrition program, say a school-feeding program for a whole country, without feeling that he is acting in a schizophrenic fashion. All intermediaries are there between pure research and very general application.

We can hope that nutrition can be an example to other sciences in translating basic knowledge to practical use in the service of mankind.

FINE STRUCTURE AND PATTERN OF LIVING THINGS

By Paul A. Weiss

Life, even in its most minute mechanisms and presentations, does not occur in random systems. All relevant functions in life are based on arrangements in which matter is organized in specific configurations we call structure. The destruction of the configuration abolishes the orderly function.

We speak of living matter. That is the wrong expression. We are dealing with living systems. The basis of their systematic nature is the non-random, methodical arrangement of parts in space and time. This does not apply to any particular size. The principle can be demonstrated through the whole gamut of size orders.

Let us draw an example from a large familiar level—the chick embryo as it is studied in the laboratory: first, the embryo in its recognizable form held in a glass container with a certain amount of fluid; second, after it has gone through the laboratory procedures of homogenization; and third, after it has been fractionated by high centrifugal force.

In some ways the homogenized embryo is very much like the embryo as we first see it. There is no change in the inventory of molecules or macro-molecules or even particular portions and no loss in weight or mass. The definite loss is in structure.

The third version—the fractionated embryo—is in some degree

Paul A. Weiss is a member and professor of the Rockefeller Institute in New York and head of its Department of Development Biology. His scientific activities extend over a wide spectrum of the biological sciences from basic problems of growth, differentiation, regeneration, and the submicroscopic organization of tissues to the development and function of the nervous system and behavior. He is a native of Austria.

of order. The particles have been sorted out from the suspension into different fractions. This is an artificial order. It compares to the chick as a store of spare parts would compare to an automobile. It is all there on the shelves. But it doesn't work. It doesn't run.

With present techniques it is easy to fractionate the chick embryo. This is a very nice technique to catalog what there is in an embryo. Inventories of chemical systems are a necessary part of life science. Progress in biochemistry in this field has been truly miraculous. But it is only one side of the story. We have not yet managed to get back the structure. And I suppose we never will.

The way chemical systems are arranged and put together is really the missing link to understanding life. One reason this holds so much promise in biology is that we know so little about it.

The principle of fine structure—organization of living systems in specific configurations—applies through the whole order of magnitudes down to those that can be measured only in angstroms (a unit of one-tenth millionth of a millimeter or four one-billionths of an inch). The hemoglobin molecule is an organized structure with dimensions of 153 times 150 angstroms. Viruses—tobacco necrosis, foot-and-mouth disease, and sylvan jaundice—are among the living organized structures that can reproduce in contact with other appropriate living systems.

We have order in each of these magnitudes. The molecules are not just mixed. They have definite arrangements. Each has an inner and outer side and a front and a rear. That goes for molecules, molecular aggregates, for the particulates which the molecules build up, the viruses, the cells, the tissues, the organism, and the community of organisms.

To understand any structure, one must know the arrangement as well as the substance. It doesn't tell you much about the

structure of a building to say it is concrete or steel or glass unless you know the arrangement.

To make the distinction between substance and patterns more clear, let us consider four biological systems: a coral colony, the scale of a butterfly wing, the spongy part of a bone, and the cuticle of an earthworm.

The coral colony builds a nicely ordered lacy grid of calcium. You find a similar pattern in the scale of a butterfly wing built up from chitin. The substance is arranged with major ribs running lengthwise and with cross-links in a mechanical structure very much like a bridge.

We find calcium again—the calcium salt, hydroxyapatite, in this instance—in another pattern formed by the spongy parts of bone. The lacy trabeculae define two linear systems that intersect at right angles. These are called orthogonal systems. The arrangement of the crystals of hydroxyapatite into a higher order system makes the bone what it is.

We find a similar pattern in the cuticle of an earthworm. It is made of chitin, the same substance as in the scale of the butterfly wing. But the arrangement is different. Instead of a grid, here the chitin is arranged in a beautiful pattern of lines intersecting at right angles as in a grate.

Super-molecular systems assume even higher complication when we step up to the cell. The thousands of little filaments produced on the margin of a cell have a standard spike-like construction. Each is about 1000 angstroms across. That is, it takes ten thousand of these little filaments side-by-side to make a millimeter.

The filaments are not built of a single material as a sculptor would build a statue of clay or marble by simply carving it out. The chemical structure of the filament is different on the inside and on the outside. Each has definite organization.

In the cell surface we have discovered a series of hold-fast mechanisms by which an epidermal cell attaches itself to the underlying substratum.

Cells have a lot of these sub-microscopic organelles. These super-molecular composites have a definite standard organization, size, and chemical make-up. They are not just substance.

The most beautiful example, perhaps, can be seen in the cilia, the flickering locomotor apparatuses of many cells from the lowliest plants and protozoans up to our own trachea.

Each cilium, each little locomotor apparatus, is built in cross-sections out of a regular array, doublets or triplets of nine fibrils in a sheath, set around a central pair. And these are arranged in rows to form a grid of still higher order.

We find similar arrangements in the interior of the cell. The cytoplasm is pervaded with a system of sacs called an endoplasmic reticulum. The sacs are often arranged concentrically. They are not static or stable. The cell moves all of the time and the sacs move, as people move in a crowd, but the pattern remains.

We have to assume a progressive complexity in molecular arrangements to explain what we see in the electron microscope.

For example, mitochondria—the power plants associated with the respiratory activity of the cell—are believed to have a laminated structure. The protein molecules are stacked in a particular way. Each molecule has its place where it sits in orientation. A similar laminated structure describes the molecular construction of the chlorophyll-carrying bodies of the plant cells.

One more example can be found in the elements of muscle teased out in meat. Inside the fiber are sub-units. When we magnify these on the electron microscope, we find each divided into still smaller units. And if we enlarge this further, we find a beautiful hexagonal array of little cylinders. These are apparently the active contractile elements. They probably consist of actin, a protein fiber running lengthwise and wrapped, in certain areas, by a jacket of myosin. The two together represent the contractile machine of the muscle.

We find random arrays of molecules in solutions that are pro-

duced artificially to study chemical reactions. But that is not the way it works in the body. Molecules in living systems appear in definite, systematic arrays or groupings. They are clearly sorted out, one from the other. Only in this fashion do we build up the relevant fine structure and eventually total structure of a living system.

How do patterns of this kind form? Some recent experiments, largely the work of an M.I.T. group around F. O. Schmitt, have clarified the problem of the structure of collagen, the fiber base of glue.

The fiber, in this instance, is built of numerous bundles of protein molecules. These line up in tandem fashion, end-to-end like a railroad train, with a periodicity of about 640 angstroms. Individual molecules are 10-13 angstroms across. They line up —each joining with its like—to form fibers of standard sizes with all unit segments in register, in cross-striation.

Early in this century, a French histologist found that you can dissolve collagen in strong acids and then precipitate the molecular chains by dialysis against a salt solution. In this way you can reconstitute the orderly arrangement outside of the body. We have a reconstituted collagen that has been completely dissolved and rearranged merely by the addition of a salt. This proves one thing. The higher order arrays, at least in collagen, are something that arises from the free interaction of molecules of specific properties.

The capacity to build up these higher order chains is inherent in the constitution of the individual unit. When the units come together, they fall into place, line up end-to-end, flank-to-flank, with corresponding segments to yield essentially the same patterns they had originally in the body. We see active synthesis of order from the individual elements right under our eyes.

It has been possible to reconstitute collagen in different periodicities than 640-angstroms. The artificial patterns have been

produced by different salt concentration, ionic strength, and the addition of adenosine triphosphate (ATP). This makes it possible to study the next higher step—the composition of a higher order system, which we find in the bone.

How does the calcium salt, hydroxyapatite, bind with the collagen framework?

Crystals actually link up chemically with the collagen fibers inside of the fibers. That is how it confers tensile strength and resistance to the bone.

When the hydroxyapatite first appears in the embryonic development of bone, the centers of crystallization appear exactly spaced with the bands of collagen fibers. There is something special about the spaces at the 640-angstrom distance which causes the hydroxyapatite to settle there.

Glimcher has recently studied the same problem. He put collagen fibers of different periodicities into solutions, which contain all the necessary calcium salts to mineralize bone. He found that mineralization would occur only if there was a guiding fiber with 640 angstroms, the natural period of collagen.

That means there is something pre-established in the crystalline system of the hydroxyapatite, which is prematched to combine in the proper spatial constellations with another system, which has a conforming constant of 640 angstroms. That is one of the basic principles by which such different systems as collagen and calcium can be combined into still higher units.

I now go from the collagen fiber to the next higher step of synthesis and show how collagen is actually being used in the body.

During the past eight years I have been making an elaborate study of the membrane under the skin of an amphibian larva. Between the top layer of the skin and the underlying loose connective tissue is a membrane of about four micra thickness (four-thousandths of a millimeter), which consists of a matrix, an

amorphous ground substance of muco-polysaccharide, in which collagen fibers are imbedded. The latter are arranged in layers like plywood, their directions alternating from one layer to the next by an angle of 90 degrees. The collagen fibers are cylinders, 500 to 600 angstroms across, lined up in this marvelously regular grid with a right angle intersection.

How does this regular structure come about? We have sought the answer to this question by simply making a hole in the membrane and watching its reconstitution.

After awhile, cells of connective tissue arrive on the scene with immature collagen fibers. These were dumped, helter-skelter like firewood, before a door to fill the gap temporarily. Then something very remarkable happened. Within a week or two, the fibers were stacked exactly like cordwood, alternating in direction and orientation. This influence went from the epidermal cell down. One cell furnished the material and another cell furnished the pattern for rearranging the material so it became organized. After awhile the original fabric was completely restored.

There are indications that the crystalline axis of the old fibers around the wound extends itself and helps give direction to this new fibrous array.

In the early stages the young fibers are quite unorganized. After 11 days or so the fibers are still a few hundred angstroms apart. Essentially what happens is that the fibers with their five- to six-hundred angstrom periodicity define a cubic pseudo-crystalline system. We obtain a new principle corresponding to crystallinity but with much larger lattice constants. Instead of the dimensions of ordinary crystals we find equilibrium points spaced at 500 to 600 angstroms. That means we are dealing with a new higher order system where certain equilibrium conditions stake themselves out in space. These are the only anchor points where the collagen chain can take hold.

It would go far beyond my present task to explain in detail how

this discovery has led to a completely new interpretation of elementary morphology in living systems.

Lest you accept too simple an explanation, I introduce you to a second set of conditions in which the right angle patterns that occur in organisms can actually be produced.

In the fluke of a whale, as a rule, the connective tissue fibers show the beautiful patterns with stress lines of tension and pressure which intersect at right angles. A cross-section of the fluke shows that it is built exactly like an aileron in an airplane.

Can patterns actually be produced in a living system by imposing external stress as an ordering element?

The structure of whale fluke gave me a clue to investigate this question more than thirty years ago.

Cells for these studies were produced in tissue culture with blood plasma as the nutrient medium. Within a few days the cells move out, essentially in random directions. The microenvironment in which the cells move and live consists of a fibrous matrix, like a sponge, and the liquid nutrient. The cells do not show any preferential orientation in this jungle. They just move around.

The long fibrous molecules of the medium, however, can be oriented in the direction of an extraneous agent when we expose them to oriented flow, stretch, or even certain high electric potentials.

The molecules, becoming oriented in their patterns as a result of the stress, then secondarily orient the cells. The cells simply trace an underlying molecular pattern and make it visible to us. It is like a logging operation on a river. The forces of the current deposit logs in parallel orientation. If the cells move in such a medium they have only one guiding pathway—the oriented path. On the less oriented parts, not under stress, they will be lost and move around in various directions.

Now an important source of orienting stresses is this: Chemical activities in the body act in a colloidal continuum. Their effects

do not remain confined to any one locality. Whatever change occurs in one part is reflected in other parts. And completely different patterns are created in a continuous substance exposed to local effects of one kind or another.

This is easy to demonstrate on glass. The deformation of a hammer blow communicates itself throughout the panes in the pattern of radial and circumferential cracks.

The same patterns can be found in the meshwork of molecules in which cells or groups of cells move and grow. An expanding network squeezes the molecules outward into a tangential, circumferential pattern. A contracting network gathers them in.

Wherever cells grow and move, the surrounding colloids lose their water. The shrinkage results in a radial pattern around growing cell colonies. That, in turn, lays down radial pathways for the cells. The chemical action of dehydration creates geometric patterns which have order in space. There are other similar situations. Each has to be studied in its own right.

On the next level of complication, the interaction of two chemical systems in a colloidal medium creates a novel pattern. If the meshes of a netted fabric are pulled through two holes the result is no longer two star-shaped radial patterns but a straight line connection between the two holes. Similarly, two cell colonies growing in tissue culture form automatically a straight track between two centers. The connections form a triangle when there are three centers. The scalar chemical actions of the cell groups thus become geometrically pattern-determining forces.

To recapitulate: we have single molecules, then those like collagen with links by which they hook end-to-end to form linear chains. An additional element of order is added when they are stretched so that all become aligned in the direction of the stretch. If there are further chemical interactions between them, the molecules slip into register and build up the oriented, segmented bundles of still higher order.

Molecules mixed in random array will arrange themselves

progressively in stacking patterns when given an interface of particular constitution—that is, the boundary between two kinds of media—and submicroscopic and finally microscopic structures such as mitochondria are formed.

Recent developments make it incumbent on us to go beyond the mere chemical composition of systems of long molecules like the proteins and to consider their orientation as of crucial importance. It appears more and more likely that practically all of the relevant communication between cells, cell organelles, and cell parts is mediated by solid-state, that is, thermally unperturbed, arrays of macromolecules combined in definitely oriented patterns. Diffusion in the ambient liquids merely provides them with the work stuffs.

For example, major changes occur in the macro-molecular network that covers the surfaces—of the cell, mitochondria, chromosome, nucleus and the like—when they are approached by a carrier of molecules with specific end groups that attract the complementary end groups e.g. of proteins in the surface. The reacting proteins will be made to stand up on end, so that the surface, which they covered more or less completely will spring a leak. And through the breach will start a secondary in-flow and out-flow of substance, electric current, osmotic activities of much greater momentum than the initiating specific stimulus carried.

Communication is mediated by this type of oriented channels. They lay down a preferential pathway for the transfer of substance as well as of current, and perhaps, as has been suggested recently, certain electron and proton transfer. Yet the primary action involves no chemical change, merely a change in orientation.

Without going into details, I predict we will find close analogies, probably homologies, for biological mechanisms in modern solid-state physics and the theory of semiconductors. In my view this indicates one of the ways biology is heading.

Now consider the next step of complication in the building up

of an-ever-more complex pattern. A cell source along a track might stream out and build up a cord or line of cells. The breaking up of that line into chunks—such as we find in the localization of feathers, hair, or scales on their linear tracts—is frequently due to a second system of lines intersecting with the primary system as in the membranes described before.

Whereas one line system is the traffic route for cells, something else is transported along the intersecting pathways. Whether this is a chemical or some other influence, we have not yet been able to identify. It produces certain threshold conditions at the nodal points where the two line systems intersect. As a result, a secondary process gets started, which leads to the formation of a hair or a feather in this particular spot. That explains why feathers and hairs come in tracts with definite geometric patterns.

One more example of the super-molecular field can be seen in the pattern of the taste papillae on the tongue. Each is a multicellular organ. The complication is that you not only have to explain the nice geometric grid but the regular alternation between different kinds of organs.

Why do we always have two kinds alternating in regular distribution patterns as in the taste papillae, the rods and cones in the retina of the eye, and many other features of biological systems?

We are just beginning to find avenues to approach this problem—to anchor biological experience to what we now know about macro-molecular systems.

We know that primary patterns of an organism change and become distorted by secondary growth. The bands in the butterfly wing were originally line patterns. They have become distorted by the progressive stretching of the wings along the veins.

We are learning to carry back our complex patterns of the organs to the simpler conditions when they are formed.

What we are dealing with—from the molecule all the way up—

is not what is present but what structure does it have that gives it opportunity to operate, to act. Structure is the guiding element in defining what actually happens among the innumerable chemical processes and events that could conceivably happen.

Structure is partly limiting the opportunity of a system. It is partly fostering certain reactions to the exclusion of others. Unless we understand structure we will not understand what makes life.

THE ORGANISM AND THE ENVIRONMENT

By Arthur D. Hasler

I will discuss interactions in nature as shown by recent findings in three lines of research: the studies of Dr. John T. Curtis of the University of Wisconsin on the distribution of plants in nature; the laboratory experiments by Dr. Thomas Park of the University of Chicago to study competition between populations of beetles; and my own research on the migration of fishes.

Distribution of Plants

Professor Curtis studies the changing patterns of communities of woods. He analyzes the mixture of species that make up a forest by measuring: (1) the importance value of each plant in the stand, and (2) the place of individual stands in the vegetational continuum.

In the upland forest of southern Wisconsin only four tree-species—black, white, and red oaks *(Quercus veluntina, Q. alba,* and *Q. rubra)* and sugar maple *(Acer saccharum)* commonly attain high importance values.

Stands in which black oak is dominant contain decreasing quantities of white oak, red oak, and sugar maple in that order. Conversely, stands with sugar maple as the leading species contain decreasing amounts of red oak, white oak, and black oak.

In the data at hand, no two stands have the same arrangement of the four species. No natural groupings of species can be seen. The gradient ranges from abundance in one area to complete absence in another.

Arthur D. Hasler, professor of zoology at the University of Wisconsin, specializes in limnology, the study of fresh water fishes. His research has been on the orientation of fishes, the physiology of aquatic organism, fishery biology, and lake fertility.

The change in species composition on a continuous gradient is like the continuous variations of the spectrum. The conspicuously different areas of prairie, oak forest, and maple stands are like the visible extremes of red, yellow, green, or blue. They can be readily seen. The intergradations, as in the spectrum, are less easily distinguished.

A continuum index for a community of woods with pines high in importance value shows a similar range from abundance in one area to complete absence in another. When the jack pine *(Pinus Banksiana)* dominates the scene, only a few red pine *(P. resinosa)* occur. Jack pine disappears as the red pine becomes dominant. Later the red pine is displaced by white pine *(P. strobus)* and this in turn yields its dominant position to eastern hemlock *(Tsuga canadensis)*. Sugar maple *(Acer saccharum)*, which has a low importance value in stands dominated by red pine, achieves a relatively pure stand at one extreme of the continuum.

A physical factor in the enviroment—in this instance the amount of calcium in the soil—can be used as a correlate. The continuous change in the amount of calcium in the soil is correlated with the change in the frequency of occurrence of the trees and the density and size of the tree population. Calcium is low where the jack pine is most important and where oaks first occur. Sugar maple increases in abundance as the amount of calcium rises in the soil.

Which is cause? Which effect? We know the sugar maple "pumps" up calcium from the deep soil. Consequently, the falling leaves enrich the soil with calcium. One might assume that the community has prepared the soil for its own better existence. One might also conclude the reverse to be true, that trees grow there because of the approximate concentration of calcium.

I quote from Dr. Curtis:

"Each species grows here and when it does on the basis of its own environmental requirements and its own past history. No

two species described have exactly the same requirements or background and no two species have been shown to grow together in, and only in, the same assemblages. The communities as we know them are chance gatherings of unrelated individuals. Each concrete example of a community is composed of a momentarily distinct and limited set of species. They are arranged in a unique spatial pattern with a never-to-be duplicated combination of numbers of individuals. The chance gatherings are not wholly random events. Rather they follow a broad pattern imprinted by the environment.

"Only a limited portion of the flora in any region possesses the proper adaptations to grow together at a particular time and place. Rarely, if ever, do all members of this limited portion actually occur together. Chance happenings of a historical nature have usually acted to prevent some of the potential members from reaching the community.

"It is the task of phytosociology to describe the combinations of plants that occur in each region of the world, to find how they came into being and how they maintain themselves, to relate them to their physical environment, and to reach an understanding of the material and energy changes which occur within them."

Competition in a Micro-Community

The example I have chosen from the extensive research of Dr. Thomas Park of the University of Chicago is a study of two species of bettles *(Tribolium confusum* and *T. castaneum).*

The beetles, raised in containers of flour, were subjected to three levels of temperature (34°C, 29°C, and 24°C) and two levels of relative humidity (70 percent and 30 percent). Differences in the ability to maintain population can be seen in the averages for three temperatures at different levels of relative humidity. At 70 percent relative humidity the mean population for *T. castaneum* was 44.50 percent, for *T. confusum,* 34.09 percent. At 30 percent

relative humidity, the mean population for *T. castaneum* was 10.34 percent, for *T. confusum,* 26.47 percent.

This study of competition between species demonstrates a fundamental problem in biology. The beauty of the model is simplicity and the possibility of controlling the environment. The living space is constant. Mortality rates and recruitment can be assessed. Problems in such models may have a bearing upon man who lives in groups competing for food and space. Dr. Park believes that:

"Research in laboratory population ecology should take its orientation from some phenomenon known or suspected to occur in nature and to have ecological consequences. Its objective is not to erect an indoor ecology. Rather it is to illuminate conceptually the general problem to which it is addressed. The research is the handmaiden of field investigations not the substitute. Findings from such studies are models of selected events in natural events. The models, though not simple, are simplified. They are under a regimen of planned control. The intrinsic interactions are likely to be intensified. To this end they are unrealistic. But they remain, nonetheless, quantitative biological models, and their unrealistic aspects may be a virtue instead of a vice. This is to say, they can contribute to the maturation of ecology, at least until they are no longer needed."

The Migration of Fish

Homing in migrating fishes such as the salmon may be defined as a behavior pattern in which an animal returns to the locality of early life after journeys of long or short duration to areas where the environment is drastically different.

We have comparatively good evidence for homing in Atlantic salmon *(Salmo salar),* five species of Pacific salmon *(Onchorhyncus kisutch, O. nerka, O. gorbuscha, O. keta,* and *O. tschawytscha),* and the steelhead or rainbow trout *(S. gairdnerii),* whose return in large numbers to their parent stream to spawn is spectacular.

In short, salmon spawn in freshwater streams and spend several

years (two to seven, depending on the species) at sea, until they reach sexual maturity. Generation after generation, families of salmon return to the same riverlet so consistently that populations in streams not far apart follow distinctly separate lines of evolution.

During a spawning movement into a river system, most of the fish swim upstream until they locate their home creek where they spawn. Adults in the genus *Onchorhyncus* die after spawning. Adults in the genus *Salmo* have been observed to spawn in subsequent years.

The most overwhelming evidence for the precision of homing in salmon is that reported in 1939 by W. A. Clemens, R. E. Forrester, and A. L. Pritchard. They marked nearly 470 thousand fingerlings in a stream of the Fraser River system and recovered almost 11 thousand of them when the salmon returned from the sea.

All marked fish were captured in the stream of their origin. Other workers report some straying. This is as it should be in a biological system. One marvels at the accuracy of the majority. There appear to be advantages of distinct value for survival in this drive to return home.

Only a few individual salmon have been marked in the home stream, caught, and remarked at sea, and recaptured in the home stream. The Oregon State Game Commission reports a series of recaptures worthy of note:

In April of 1958 Commission workers marked steelhead fingerlings by removing both ventral fins and the adipose fin and released them from the Alsea River hatchery on the central Oregon coast. The following September one of the fish, by then 365 mm. long, was captured 75 miles southeast of Geese Island, southwest of Kodiak Island, Alaska. It was marked with an identifiable tag. In February 1960 the fish, 558 mm. long, was recovered at the Alsea River hatchery.

Two other salmon in the group of 59 tagged on the high seas have been recovered in Washington—one in the Samish River and the other on the coast.

Fishery biologists from Japan, Canada, and the United States, add, each year, to our knowledge of fish routes at sea. The international North Pacific Fisheries Commission recently noted that the red salmon of Bristol Bay feed in great numbers around Attu Island, 1200 miles away. A great number of tags from that district have been retaken in North American estuaries, particularly Bristol Bay but not in the Gulf of Alaska. Pink Salmon spawning in Kamchatka have been tagged all along the Aleutian Islands as far as 1200 miles from their own streams. The chum salmon returning to the Okhotsk Sea have been tagged 1700 miles from their home streams and so have chum salmon spawning in Hokkaido. Kings and steelheads, tagged near Adak Island, have returned to the Columbia River system, a migration of 2500 miles; one was recaptured in the Snake River, Idaho.

L. R. Donaldson and H. R. Allen confirmed earlier work that showed homing ability is the result of some sort of "imprinting" by the environment. They switched salmon stock and let the eggs develop in different waters. Adult fish returned from the sea to waters in which they hatched and lived as fry, not to the stream of their parents. Donaldson used this finding as a basis for building a run of salmon to a newly built hatchery at the University of Washington.

What cues and mechanisms guide these fish in their migrations? How do salmon recognize the main river as well as the home tributary? How do they negotiate the route at sea without visual landmarks?

I have proposed the hypothesis that young salmon are "imprinted" by an organic odor of the home stream in the early fingerling period. Each stream acquires a different odor, perhaps derived from a community of plants or their decomposition prod-

ucts in the stream or drainage basin. The mature salmon, returning from the sea, swims upstream in response to the water current. It rejects tributary after tributary until it detects traces of the home stream. It may make faulty choices but continues the search after backtracking—a behavior pattern frequently observed. The salmon is stimulated to enter the home stream by the characteristic odor.

I do not agree with the hypothesis that an animal "follows a gradient." If an animal stayed in the gradient, it would soon become adapted to the odor and would be incapable of responding to it. I think it more likely that the salmon's behavior during the ascent of the river system is similar to that of a dog following an odor track. The dog does not stay exactly on the track. Instead it crosses back and forth, responding to the presence and absence of the scent as it follows its prey.

I believe the salmon is guided home by an odor that remains constant from year-to-year and has meaning only for those salmon conditioned to it during their fresh-water sojourn.

The theory presents three distinct problems: (1) Do streams have characteristic odors to which fish can react? If so, what is the nature of the odor? (2) Can salmon detect and discriminate between such odors? and (3) Can salmon retain odor impressions from youth to maturity?

W. J. Wisby and I answered the first question in an experiment in which we trained a group of blunt-nosed minnows to discriminate between the waters of two chemically different Wisconsin creeks. We proved that scent-perceiving organs were the sole means of discrimination in these tests. When we destroyed the olfactory tissue of the trained fish they no longer responded to the training odors.

Chemical analysis of the stream waters indicated that the main difference between them was in the total organic fraction. We obtained evidence to substantiate this by separating the water into

various fractions and presenting these to the fish. Those trained previously to react to natural water did not react to the redissolved inorganic ash or to the distillate or residue of water fractionated at 100°C. They recognized the distillate, however, but not the residue of water fractionated by vacuum distillation at 25°. This is a strong indication that the odorous stimulant is a volatile organic substance.

A test of the retentative capacities of the trained minnows showed that the fish could differentiate between the odors for a comparatively long period after the training ended, particularly if the learning took place in the early life of the minnow.

Next, we trained salmon fingerlings in the laboratory and found that they too could discriminate between the odors from two Wisconsin creeks. Then in the field we captured sexually ripe coho salmon *(O. kisutch)* at two branches of the Issaquah River in Washington. We plugged the nasal sac of half of the 302 specimens with cotton before returning all of the fish downstream to make the run and selection of stream again. Those with plugged nasal sacs returned in random fashion. The great majority of those from the other group again selected the stream of their first choice.

We propose to test our hypothesis further by using an artificial substance by which salmon fry can be conditioned in a hatchery or spawning riverlet. The substance would then be used to decoy them on their return into riverlets downstream from the site of conditioning.

The odor must be neither a repellant nor an attractant for the unconditioned salmon. One of my students has suggested that the compound, morpholine, might fit the requirements. It is soluble in water, detectable in extremely low concentrations, and chemically stable under stream conditions. It should influence

behavior of only salmon previously conditioned to it because, in low concentrations, it neither attracts nor repels salmon unconditioned to it.

Field tests may now be made to find whether salmon conditioned to morpholine in a hatchery or stream can be decoyed to a stream other than that of their birth when they return from sea. It would be instructive to know whether the "memory" of an artificial odor can be superimposed upon the natural odor of the stream.

Recently, H. Teichmann has found the olfactory acuity of the eel for pure chemicals to be remarkably high. Young eels, conditioned by training, detected concentrations of 3 x 10-20 of beta-phenylethylalcohol. The dilution of 1 millimeter of the compound to this concentration would yield a volume approximately 58 times the volume of the Lake of Constance (the Bodensee). The amount of the chemical in the eel's olfactory sac at this concentration would be only two or three molecules.

The principal role of odor in the ocean, it seems to me, is to give the fish a signal for home recognition. Fish swimming within a water mass would have no sense of being displaced as the mass moved unless there were fixed visual or tactile features in the environment (compare the experiences of balloonists in a cloud). Where two water masses meet, fish might perceive differences in salinity, dissolved gases, and odor. Unpublished data from our laboratory convinces us that the minnow can smell the difference between water from the Georges Bank and samples from the Sargasso Sea.

When two water masses meet there might be a sliding of one over the other—a "shear effect"—that would help the fish to sense that it had reached the edge of a water mass, stimulating it either to enter the new mass or stay in the water mass where it had been swimming.

The sensing of salinity, gases, or odors at any one place at sea appears to be the signal for recognizing, for example, an oceanic spawning site rather than cues for directional orientation.

Home-finding in a stream system may depend upon the recognition of an odor and other yet undiscovered guideposts. It seems to me, however, that the olfactory hypothesis is inadequate to explain the movements of salmon in the ocean. Certainly other cues are used.

Our initial attack on this problem was to study a less complex type of homing than that in salmon. For a number of years my co-workers and I have studied the natural history of the white bass *(Roccus chrysops* Raf.) in Lake Mendota, Wisconsin. We have located only two major spawning grounds—Maple Bluff and Governor's Island, both on the north shore of the lake. The sites are 1.6 kilometers apart. The white bass congregate here in late May and early June when temperatures range from 16° to 24°C.

During the spawning seasons of 1955, 1956, and 1957 we captured white bass in fyke nets, marked them with numbered disk tags, transported part of the catch in open tanks to stations in the lake for daytime release, and released others on the spawning grounds.

We were impressed by the high percentages of recapture (89 to 96 percent) of displaced spawners. They returned to the original site from a release point 2.4 kilometers away. The percentage of fish recaptured and the time lapse between release and recapture were of the same order of magnitude for fish that were displaced and those released on the spawning site. This indicates an almost complete return of the displaced fish to the spawning grounds.

Subsequently, we observed the "take-off" direction of displaced white bass by attaching a plastic float to the fish and following as the fish towed it along. Our observations in 1958 and many additional releases since then convince us that the course taken upon

release on sunny days is generally north toward the spawning grounds. This "take-off" serves to bring the fish promptly to shore in the general vicinity of the spawning areas. Once there they appear to locate their specific spawning ground by other cues. On cloudy days the fish swim at random.

The white bass of Lake Mendota appeared to be able to maintain a constant compass direction in unfamiliar territory. This ability would help them to arrive at the shore directly and avoid random wandering in the open water.

Our next task was to explore the possibility that a sun-compass mechanism could be helpful to fish in open-water migration.

We tested the fish under the open sky in a specially designed tank. Our method relied upon an escape or cover-seeking response which could be scored.

The tank had 16 small compartments arranged around a circle. None of them could be seen by the fish from its starting point in the middle of the large tank and only one of them was open. The others were covered by a metal band. In training tests, at frequent intervals, the fish was released from a cage in the center of the tank and given a small electric shock to make it seek cover in the small open compartment which was always in the same compass direction. When the fish had learned the location of the training compartment, we conducted tests in which all 16 compartments were open. Usually the fish chose the compartment in the compass direction in which it had been trained to seek shelter.

Trained fish tested under completely overcast skies were disoriented. This showed that the sun was the fish's point of reference and that the fish had learned to seek cover in the same direction at different times of days. It allowed for the movement of the sun.

Our crucial test substituted an artificial "sun" indoors for the actual sun. A sun-compass fish responded as though it were responding to the actual sun at that time of day. It chose a hiding

box of the appropriate angle to the artificial sun. Hence, the existence of an orientation rhythm, which is associated with the so-called "biological" clock, has been established.

Field and laboratory evidence on four different species of freshwater fish make it clear that many fish possess a sun-compass mechanism. Our unpublished laboratory tests on young silver salmon show they too are oriented by this mechanism.

We cannot stop here. A salmon with a sun-compass mechanism needs other sensory and physiological abilities to accomplish its migratory feat.

It now becomes imperative to make field studies of migrating salmon at sea. Sexually mature salmon should be captured in purse seines near the mouth of a home river, equipped with tracers similar to those used on the white bass, and displaced several kilometers out to sea, where their "take-off" and swimming direction can be charted.

Preliminary studies suggest that the altitude of the sun plays a role in orientation. H. O. Schwassmann and I trained sunfish *(Lepomus cyanellus)* in orientation to the sun at Madison, Wisconsin (43°N) and then took them and our circular sun-orientation maze to Belem, Brazil (1°S). When the fish were tested where the sun appears to move counter clockwise, they continued to compensate for the azimuth curve of the sun that was "correct" for Madison.

Under the sun at the equator the oriented behavior of the displaced fish rapidly deteriorated. All of them showed an increasing tendency to maintain the same angle to the azimuth position of the sun throughout the day.

What would have been the response of a mature salmon that moved from one latitude to the other at a normal and gradual rate?

Other studies must be undertaken to solve the riddle of the salmon's migratory accomplishments. One possibility is that as

the salmon reaches maturity it accumulates a certain temperature budget within a water mass where it swims. When this budget is exceeded the fish takes another angle from the sun to correct its course and keep from drifting too far off course. This hypothesis may be too bold but it is the kind of thinking we must do if we are to decipher the migratory pattern.

We need to know more about the movements of salmon at night. Clifford Barnes of the University of Washington observed salmon migrating at right angles to his oceanographic research vessel in the northeastern Pacific. A school of large salmon could be seen clearly in the luminescent sea as they swam on a fairly straight course until they were out of sight. We urgently need a technique for tracking them to gain knowledge of their night activities at sea.

The International North Pacific Fisheries Commission has increased efforts to capture and mark thousands of Pacific salmon on the high seas. Japanese and North American workers are accumulating field data rapidly and the prospect is that there will soon be enough information to plan studies of the oceanic movements of salmon, eels, tuna, and other fish that migrate long distances.